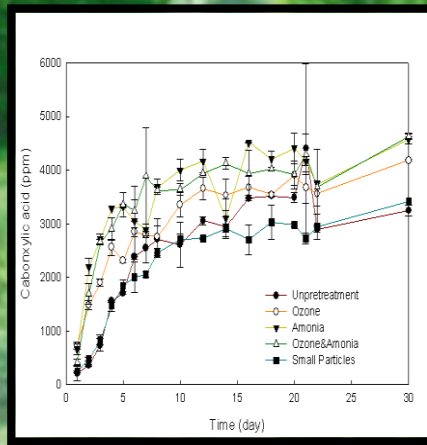
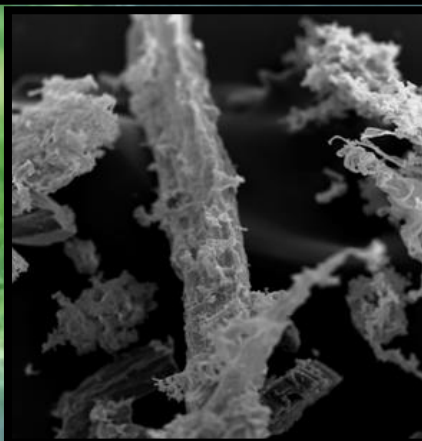


**2011–13
Biennium**

Anaerobic Digestion-Related Research Summary for WSU ARC/WSDA - Appendix A Funds



Craig Frear, Manuel Garcia-Perez, Chad Kruger, Shulin Chen



June 30, 2013

Anaerobic Digestion-Related Research Summary for WSU ARC/WSDA – Appendix A Funds

by

Craig Frear, Nicholas Kennedy, Quan-Bao Zhao, Jie Liu, Baisuo Zhao, Jingwei Ma, Liang Yu, Mahesh Bule, Shulin Chen, Allan Gao, Manuel Garcia-Perez, Matthew Smith, Waled Suliman, Changle Pang, Sylvia Kantor, Georgine Yorgey, and Chad Kruger

Washington State University

Applied Bioenergy Research, also known as *Appendix A* research

In the 2007–09 biennial operating budget the Washington State Legislature appropriated \$2 million to Washington State University to provide a pool of funds that the University and Washington State Department of Agriculture were to jointly target to applied bioenergy research. The funds were requested by WSU and WSDA to undertake near-term, applied research needed to successfully implement the Energy Freedom program and bioenergy initiatives enacted in 2006. Examples of projects to be funded were listed in *Appendix A*, which was attached to the funding request.

Since 2007, WSU's Agricultural Research Center and WSDA have collaborated on this research effort. The *Appendix A* funds have been directed to research projects coordinated by the WSU Department of Crops and Soils, as the Biofuels Cropping Systems project, and to research projects coordinated by the WSU Department of Biological Systems Engineering, in the area of energy conversion from agricultural wastes.

Due to stresses on the state budget, the allocation for Appendix A research was reduced. During the 2011–13 biennium, more than \$1.2 million was available for Appendix A research with \$590,000 directed to anaerobic digester-related research.

For more information about the Applied Bioenergy Research project, contact:

James W. Moyer
Associate Dean for Research,
College of Agricultural, Human, and Natural
Resource Sciences
Director of the Agricultural Research Center
PO Box 646240
Pullman, WA 99164-6240

Mary Beth Lang
Bioenergy & Special Projects
Coordinator
Washington State Department of
Agriculture
PO Box 42560
Olympia, WA 98504-2560

Executive Summary

Washington State University, in partnership with the Washington State Department of Agriculture, has conducted targeted applied research and extension work related to anaerobic digestion and other energy conversion technologies for dairy, animal and organic wastes since 2007. During the 2011–13 biennium, research and extension activities were designed to address the present and future technical needs of our dairies, emerging clean technology industries, and rural communities. This report summarizes the major results and conclusions from work in eight general areas.

1. Nutrient recovery from dairy manure

Nutrient recovery has the potential to provide dairy farmers with an economically viable option for reducing negative air and water quality impacts from current manure management practices. Various technologies are in development in the United States, each with their own opportunities and challenges. Work in this area focused on two parallel efforts: a more established effort to commercialize a novel nutrient recovery technology that is incorporated within an anaerobic digestion platform, and a newer effort to explore opportunities to incorporate nutrient recovery into the bio-char platform.

Within the anaerobic digestion platform, significant progress has been made over the last two years towards commercializing the novel nutrient recovery technology developed at Washington State University. Currently, three dairies and one poultry facility are using this technology at commercial scale. While these demonstrations have shown technical and economic hurdles that still need to be overcome, this represents the greatest number of facilities using any combined nitrogen and phosphorus nutrient recovery technology within the United States.

Within the bio-char platform, Washington State University researchers investigated several specific strategies aimed at improving the ability of bio-chars to recover phosphorus and nitrogen from dairy liquid wastes. First, they explored whether adding iron or calcium to bio-chars produced from the pyrolysis of anaerobically digested dairy manure fiber could improve the bio-char's ability to recover phosphate from a liquid waste stream. The highest removal of phosphate, 53% after 12 hours, resulted when calcium was added to the dairy manure fiber prior to pyrolysis. A separate set of experiments was conducted to examine the effect pyrolysis temperature had on oxygen complexes formed on bio-char surfaces by post-pyrolysis air oxidation, with the best results obtained at 250°C. These oxygen complexes are an indicator of ammonium removal capabilities as shown by experiments that determined a linear correlation between oxygen complexes and ammonium removal capabilities.

Using the data collected in these experiments, researchers determined the approximate amount of phosphorus that could be removed from liquid wastes on a hypothetical 1,000-cow dairy operation using a bio-char filtration system deployed via a truck trailer. Results indicated that approximately 1 to 5 tons of char per day would be required assuming a concentration of 50–100 mg phosphorus per liter within the wastewater. However, if all the phosphorus is assumed to be ionic phosphate, approximately 10 to 20 tons of bio-char per day would be required.

Similar estimations were made of the amount of modified char required to treat dairy effluent at various total ammonia nitrogen loadings at a hypothetical 1,000-cow dairy operation, assuming an upper limit of 1.5 g/L ammonia in effluent, with 200–500 mg/L after nutrient recovery. Results indicated that 5 to 40 tons per day of oxidized char would be required to treat the effluent produced, with a maximum of 115 tons. Though the lower range is reasonable, the upper range would likely lead to significant material handling difficulty. An additional concern is competitive inhibition of ammonium recovery by sodium and potassium, both of which are present in significant quantities.

Based on these results, additional work needs to be done before this is a viable technology. Future work should focus on achieving ideal conditions for the generation of high surface area and high acidity chars at pyrolysis temperatures between 550 and 600°C and oxidation for less than one hour at a temperature of approximately 250°C. Any filtration system developed will depend heavily on the final concentration of total ammonia nitrogen requiring filtration and the concentration of competing cations such as sodium and potassium present in the effluent.

2. Anaerobic sequence batch reactors

Due to the large amount of added water, flushed dairy manure requires larger digesters than scraped manure to treat wastes from an equivalent number of cows, raising costs. To address this, various techniques have been developed to reduce the substrate volume by separating the liquid and solid fractions. Unfortunately, this results in a loss of up to half of the organic matter, reducing digester performance. An alternate approach to minimize the required reactor size is an anaerobic sequence batch reactor approach, developed at Washington State University. By utilizing dairy manure fiber as a bio-film, biogas production is enhanced and a high-rate process, requiring less volume, is achieved. Results indicated that a low hydraulic retention time of 4 to 6 days and relatively high organic loading rate of 0.5–1.3 g volatile solids loaded/L/day was achievable in the anaerobic sequence batch reactor at a temperature of 22°C.

Researchers subsequently investigated the microbial community dominating at this low hydraulic retention time and temperature to better understand the factors contributing to anaerobic digestion performance. Analysis indicated the reactor had established a population dominated by *Methanosarcina*. This is notable since *Methanosarcina* are known to create a more stable and efficient anaerobic process. In addition, *Methanosarcina* are the most versatile methanogens and have a higher substrate utilization rate, growth rate and cell yield when exposed to an environment with relatively high acetate and hydrogen concentration, such as is experienced in dilute manure treated at short hydraulic retention times.

3. Biogas purification within the anaerobic digestion-nutrient recovery platform

In the Pacific Northwest, low received electrical prices negatively impact the economics of anaerobic digestion projects. One way to improve economics and further promote the adoption of new digesters in the region is to develop simple and inexpensive ways to upgrade biogas to renewable natural gas. Consequently, biogas purification within the WSU-developed nutrient recovery system was investigated. The effluent coming out of the nutrient recovery system has been stripped of carbon dioxide via aeration and high temperature. This process increases the pH

of the effluent to greater than 9.7. At this high pH, the effluent can be used to remove hydrogen sulfide and carbon dioxide from raw biogas, upgrading it. Researchers constructed and used a bubble column reactor as the absorption column, an approach that is relatively inexpensive to build and easy to maintain. Nearly 100% removal of hydrogen sulfide was achieved by optimizing the bubble column reactor for hydrogen sulfide removal, through a combination of large bubbles, high flow rate, and low effluent height. Commercialization of this new technique for the complete removal of hydrogen sulfide is currently underway with the assistance of DVO Inc., with the ultimate goal of removing both hydrogen sulfide and carbon dioxide from raw biogas.

4. Anaerobic digestion on small dairies

Unfortunately, anaerobic digestion based on the traditional business model of electrical generation is not currently economically viable for small-scale dairies. In this project, researchers installed and tested a new digester design on Cherry Valley Dairy, a small-scale milk producer/processor operation located near Seattle, Washington. As Cherry Valley Dairy transitioned to a producer/processor model, it wanted to know whether anaerobic digestion could improve waste management and sustainability of its operation, while reducing odors and generating renewable energy. To meet these needs, Washington State University and Andgar Corporation designed a new anaerobic digestion reactor. Instead of utilizing an expensive engine to produce electricity and heat, the biogas was used to produce thermal energy for heating and chilling of Cherry Valley Dairy's new micro-processing facility via a low cost boiler and condenser. The unit was installed and, by mid-July of 2012, was producing biogas and operating at a temperature of around 38°C. As of March 2013, the unit was making approximately 100 cubic feet of biogas per day. Unfortunately, the biogas was of poor quality, containing less than 10% methane. The team is currently seeking additional funding for further evaluation of management, financial and environmental implications.

5. Pretreatment of fibrous feedstock for entry into digester

Pretreatment is one way to improve the digestibility of cellulosic material, including dairy manure and lignocellulosic wastes such as lawn clippings. Improving digestibility through pretreatment could improve digester efficiency and raise biogas production. In this area, researchers investigated the effects of three pretreatments on grass samples: soaking aqueous ammonia pretreatment, ozone pretreatment, and the combination of soaking aqueous ammonia and ozone. Enzymatic hydrolysis showed that the highest sugar recovery (~ 90%) resulted from the combination of the two pretreatments. Even though the soaking aqueous ammonia did not achieve the highest biogas production, the authors tentatively recommend this pretreatment as the best option for pretreating dairy manure fiber, based on the fact that this process does not require any special handling, as well as the potential for synergies when integrated with the WSU-developed nutrient recovery system. Further techno-economic analysis will be required to support this conclusion, and additional experiments are planned to see how this pretreatment affects the anaerobic digestion of dairy manure fiber.

6. Anaerobic digestion of algal biomass residues with nutrient recycling

As interest continues to intensify in lipid extraction from algae for energy production, there is a parallel interest developing in anaerobic digestion of algal residue left over after lipid extraction.

Scientists at Washington State University, working alongside scientists at the National Renewable Energy Laboratory, investigated the biochemical methane potential of five different algal strains.

The studies performed at Washington State University resulted in a number of key findings. One unexpected conclusion was the significant inhibitory effect that the solvent chloroform had when the lipid-extracted biomass was anaerobically digested. In the most extreme example, use of the solvent chloroform/methanol resulted in almost no methane production when the algal biomass was digested. In addition, a linear relationship between specific methane productivity and ash-free lipid content was determined, showing the strong relationship between long chain fatty acid content and methane production. This may reduce the future need for costly and time-consuming methane potential studies on microalgae biomass. Another important parameter that affected biogas production was the inoculum-to-substrate ratio. As this ratio increased, so did the biogas production; though, the presence of long chain fatty acids had inhibitory effects on the biogas production. This provides further evidence that long chain fatty acids should be monitored for successful digestion of algal biomass.

7. Extension and support for transfer of anaerobic digestion technologies

Extension and support is instrumental for the continued adoption of anaerobic digestion in the region and the development of the anaerobic digestion industry. Dr. Frear continued to lead project development, *Pro Forma* analysis and pre-engineering efforts for several projects including the PacifiClean and Outlook POD projects in Washington. He also continued to explore more sustainable ways to produce desired nutrient recovery co-products with the help of industry leaders, scientists at the Washington State University Prosser campus, and farmers. Lastly, Dr. Frear attended and presented at 17 conferences during this biennium to promote the adoption of anaerobic digestion and nutrient recovery.

Another important aspect of work in this area was providing information to governmental leaders and others on anaerobic digestion and nutrient recovery. With this goal in mind, the extension team produced a nutrient recovery webinar, an anaerobic digestion/nutrient recovery field day, a nutrient recovery fact sheet, a financial fact sheet, an anaerobic digestion project overview video, and an anaerobic digestion workshop at the Bioenergy Research Symposium. This strategy combined “in person” opportunities to ask questions and generate discussion of issues, along with “durable” products such as the video that individuals can return to over time, or use in their own work. Each of these educational tools aimed to enhance understanding of anaerobic digestion and nutrient recovery. This included general information on the rationale and more in-depth information on specific topics (e.g. economics, various technologies, end use of products). Hopefully, these efforts will provide policy makers with a better understanding of the positive impacts anaerobic digestion and nutrient recovery can have on waste management, renewable energy generation, and nutrient management.

8. Smaller projects on various anaerobic digestion topics

In addition to the seven major areas of work, researchers at Washington State University carried out several smaller projects, including explorations of (1) psychrophilic (low temperature) anaerobic digestion, (2) carboxylate platforms, and (3) methane super-saturation.

While mesophilic (moderate temperature) anaerobic digestion is more efficient than digestion at lower temperatures, it can be problematic to operate stably in regions with cold temperatures during winter months due to thermal constraints and economics. Consequently, researchers isolated high-performance psychrophilic consortia from low-temperature environments. Results indicated that the microbial consortia sampled from well-established digesters located in Heilongjiang, China showed the highest production of biogas. If ongoing microbial analysis confirms that the anaerobic bacteria found in these digesters are in fact psychrophilic, these populations will be used in ongoing work on psychrophilic anaerobic digestion.

A separate investigation was conducted to see whether or not algal biomass could be anaerobically digested to produce carboxylic acids, which are used in the production of polymers, pharmaceuticals, solvents, and food additives. The research used a mixed culture of cow rumen bacteria for the transformation of microalgal biomass to carboxylates using anaerobic sequence batch reactor technology. Production of carboxylates was impacted by the hydraulic retention time, with the highest production occurring at a hydraulic retention time of 12 days. Given that many studies have suggested that the biogas potential from algal biomass is too small to be viable, this research suggests that carboxylate salts may represent a better option. Construction of a clone library to determine the bacterial community that dominated during the 12-day hydraulic retention time is currently underway.

The final study investigated ways to reduce methane super-saturation in digester effluent. Methane super-saturation occurs when methane remains dissolved within the liquid effluent from the anaerobic digestion process. Researchers have reported that this can account for roughly 40 to more than 60% of the theoretical methane potential in the reactors. This reduces the renewable energy production and greenhouse gas mitigation impact of digesters. To investigate low-cost methods for reducing methane super-saturation, researchers utilized a vacuum pump to treat the digester effluent from a continuous stirred tank reactor. Results indicated that over 10 ml of methane/L could be removed at a temperature of 55°C. In addition, a relatively high percentage of dissolved carbon dioxide was recovered during the experiments, which simultaneously raised the pH of the effluent above 9. This is an important side effect since it indicates that the process might generate benefits if integrated with ammonia stripping. In some nutrient recovery technologies, alkali, a relatively expensive input, is used to raise the pH and enable recovery of nitrogen. An economic analysis will need to be carried out to determine whether degassing through a vacuum pump (with associated energy costs) could replace the alkali used in traditional ammonia stripping techniques.

Links to more detailed data analyses for each of these projects will be available on an anaerobic digestion website hosted by Washington State University's Center for Sustaining Agriculture and Natural Resources (currently under construction).

Table of Contents

Executive Summary	iii
Table of Contents	viii
List of Figures	x
List of Tables	xii
List of Abbreviations	xiii
Nutrient Recovery within Anaerobic Digestion and Bio-char Platforms	1
1.1 Nutrient Recovery for Anaerobically Digested Manure	1
1.1.1 Introduction	1
1.1.2 Background	1
1.1.3 Commercialization Progress and Performance	6
1.2 Nutrient Recovery within a Bio-char Platform	8
1.2.1 Introduction	8
1.2.2 Background	8
1.2.3 Methods and Results	9
1.2.4 Conclusions	15
Flush Dairy and Anaerobic Sequence Batch Reactor Approach	16
2.1 Introduction	16
2.2 Methods and Results	17
2.3 Conclusions	20
Biogas Purification within the Anaerobic Digestion/Nutrient Recovery Platform	21
3.1 Introduction	21
3.2 Methods and Results	22
3.3 Conclusions	25
Anaerobic Digestion on Small-sized Dairies	26
4.1 Introduction	26
4.2 Demonstration Site and Results	28
4.3 Conclusions	29
Pretreatment of Fibrous Feedstock for Entry into Digester	30
5.1 Introduction	30
5.1.1 Pretreatment of Lignocellulosic Material	31
5.1.2 Pretreatment and Anaerobic Digestion	33
5.2 Methods and Results	35
5.2.1 Sample Preparation and Compositional Analysis	35
5.2.2 Developing Pretreatment Technologies for Improved Enzymatic Hydrolysis	36
5.2.3 Utilizing Untreated and Pretreated Grass Clippings for Methane Production	37
5.3 Conclusions	37
Anaerobic Digestion of Algal Biomass Residues with Nutrient Recycle	39
6.1 Introduction	39
6.2 Methods and Results	40
6.2.1 Whole Cell Microalgae and Lipid-extracted Microalgae Residue	40

6.2.2 Bench-scale and Scale-up Microalgae Digestion	40
6.3 Conclusions	46
Anaerobic Digestion Extension-Technology Transfer Project	47
7.1 Assisting AD Industry Growth	47
7.2 Outreach Programs and Materials	48
7.2.1 Nutrient Recovery Webinar	48
7.2.2 Anaerobic Digestion Project/Nutrient Recovery System Field Day	49
7.2.3 Nutrient Recovery Technology Fact Sheet	49
7.2.4 Anaerobic Digestion Project Financial Trade-off Fact Sheet	49
7.2.5 Anaerobic Digestion Project Overview Video	50
7.2.6 Anaerobic Digestion Workshop at Bioenergy Research Symposium	50
Smaller Projects on Various Anaerobic Digestion Topics	51
8.1 Psychrophilic Anaerobic Digestion	51
8.1.1 Introduction	51
8.1.2 Biogas Production from Low Temperature Sources	52
8.1.3 Enhancing Biogas Production of Psychrophilic Anaerobic Digestion with Trace Elements	54
8.2 Carboxylate Platforms	56
8.2.1 Introduction	56
8.2.2 Methods	57
8.2.3 Results and Discussion	59
8.2.4 Conclusions	62
8.3 Methane Super-Saturation	62
8.3.1 Introduction	62
8.3.2 Methods	62
8.3.3 Results and Discussion	63
8.3.4 Conclusions	66
References	67

List of Figures

Figure 1.1: Integrated pyrolysis-AD systems for the production of methane, heat, and bio-char enriched in nutrients and clean water	9
Figure 1.2: Diagram of treatments to screen pre- and post-pyrolysis treatment of bio-chars	10
Figure 1.3: Formation of carboxylic acid groups under different oxidation conditions	11
Figure 1.4: Ammonium adsorption vs. total carboxylic groups from ozone oxidation	12
Figure 1.5: Portable lagoon filtering system	14
Figure 1.6: Char requirements (<i>left</i>) and concentration profile for adsorption with Ca-infused bio-char in a packed bed column (<i>right</i>)	14
Figure 2.1: Different manure management strategies available for dairy operations	16
Figure 2.2: Scanning electron microscope rod and coccoid microbial cells attached to the fiber (<i>left</i>) and fluorescence <i>in situ</i> hybridization image shows fermentative bacteria attached to the surface of fiber and methanogens distributed on the outer layer of bio-biofilm (<i>right</i>)	18
Figure 2.3: ASBR process divided into six stages to enhance the retention of biomass for improved biogas production and digester efficiency (Wang et al., 2011)	19
Figure 2.4: Phylogenetic relationship among 18 OTUs with partial 16S rRNA gene sequences of known methanogenic <i>Archaea</i> based on the neighbor-joining analysis	20
Figure 3.1: Multiple paths through which biogas can be used as renewable energy/fuel (Weiland, 2006)	22
Figure 3.2: Ammonia and P recovery system with attached H ₂ S and CO ₂ removal	22
Figure 3.3: H ₂ S removal efficiency using an airstone sparger (C ₁) and inlet orifice (C ₂); photographs were taken with a high-speed camera in water	24
Figure 3.4: Effect of effluent height (<i>left</i>) and superficially velocity (<i>right</i>) on the H ₂ S removal efficiency and G/L ratio	24
Figure 4.1: Capital cost per dairy cow for complete mix, plug flow, and covered lagoon AD systems (AgStar, 2010a)	26
Figure 4.2: Dairy digesters and dairy farms in WA, 2013	27
Figure 4.3: Small-scale vertical mixed plug flow digester with a hot water heater for heat generation located CVD near Seattle, WA	29
Figure 5.1: Schematic of the role of pretreatment in the conversion of biomass to fuel (Kumar et al., 2009)	31
Figure 5.2: The structure of a cellulosome (Schwarz, 2001)	33
Figure 5.3: Biomethane productions of different grass treatments	37
Figure 6.1: Algae cultivation with AD and nutrient recycle—either whole cell or residue	39
Figure 6.2: Relationship between ash-free lipid content and SMP for microalgae biomass	41
Figure 6.3: Biogas production curves for digestion of NS1 (A) and NS2 (B) at various I/S ratios	42
Figure 6.4: Effects of dosing Ca on CH ₄ production from NS1 (<i>left</i>) and NS2 biomass (<i>right</i>)	43
Figure 6.5: Biogas production curves from BMP tests on whole cell biomass	43
Figure 6.6: CH ₄ production during digestion of N1 treated with different solvents A: Chloroform/methanol (2:1); B: Hexane/isopropanol (3:2); C: Dichloroethane/methanol (1:1); D: Dichloroethane/ethanol (1:1); E: Acetone/Dichloroethane (1:1)	44

Figure 6.7: CH ₄ production from NS1 (<i>top</i>) and NS2 (<i>bottom</i>) digester at different OLR	45
Figure 8.1: Biogas production from 10 low temperature consortia (triplicate)	53
Figure 8.2: Biogas production of SH inoculated digester (<i>left</i>) and LD inoculated digester (<i>right</i>)	55
Figure 8.3: Overview of the CBP from algal biomass to carboxylate salts	57
Figure 8.4: Schematic of the fermentation process with attached monitoring system	58
Figure 8.5: The total acid concentration for the lab-scale ASBR bioreactor at a 12-day HRT	60
Figure 8.6: T-RFLP pattern of PCR-amplified nearly full-length bacterial 16S rRNA genes of different HRTs digested with the restriction enzyme Hae III	61
Figure 8.7: CSTR reactor with attached vacuum pump and gas collection system	63
Figure 8.8: D-CH ₄ recovery at various temperatures and mixing speeds	64
Figure 8.9: Recovery of D-CO ₂ (<i>left</i>) and D-H ₂ S (<i>right</i>) at various temperatures and mixing speeds	65
Figure 8.10: pH of the effluent at various temperatures and mixing speeds	66

List of Tables

Table 1.1: Summary of P removal and mineral leaching from various treated chars	10
Table 1.2: Annual tons of char required to remove ionic phosphorous from the effluent water of 1,000-cow dairy given different retention rates	12
Table 1.3: Annual char required to remove TAN from solution in a 1,000-cow dairy (<i>left</i>) and metal cation concentrations in digester effluent based on dairy manure (<i>right</i>)	13
Table 5.1: CH ₄ yield from the AD of different plant material (Braun et al., 2010)	30
Table 5.2: Commonly used parameters for different pretreatment techniques reported in the literature (Gao, 2012)	32
Table 5.3: Compositional analysis of untreated and pretreated grass	36
Table 5.4: Sugar recovery of lawn grass after different pretreatments	36
Table 6.1: BMP and performance indicators for studied biomass	41
Table 8.1: Biogas potential generated from different microbial consortia	52
Table 8.2: Operational parameters and characteristics of un-extracted algal biomass samples	58
Table 8.3: Operational conditions of the experiments	63

List of Abbreviations

AD	Anaerobic Digestion	NR	Nutrient Recovery
AFEX	Ammonia Fiber Expansion	NRCS	Natural Resources Conservation Service
ASBR	Anaerobic Sequence Batch Reactor	NREL	National Renewable Energy Laboratory
BMP	Biochemical Methane Potential	Mg	Magnesium
Ca	Calcium	Mn	Manganese
CAFO	Concentrated Animal Feeding Operation	O ₂	Oxygen
CBP	Consolidated Bioprocessing	OLR	Organic Loading Rate
CH ₄	Methane	OSAA	Ozone and Soaking Aqueous Ammonia
CHP	Combined Heat & Power	OTU	Operational Taxonomic Unit
C:N ratio	Carbon to Nitrogen Ratio	P	Phosphorus
CO ₂	Carbon Dioxide	PNW	Pacific Northwest
Co	Cobalt	RNG	Renewable Natural Gas
COD	Chemical Oxygen Demand	S	Sulfur
CSANR	Center for Sustaining Agriculture and Natural Resources	SAA	Soaking Aqueous Ammonia
CSTR	Continuous Stirred Tank Reactor	SEM	Scanning Electron Microscopy
Cu	Copper	SMP	Specific Methane Productivity
CVD	Cherry Valley Dairy	TAN	Total Ammonia Nitrogen
EPA	Environmental Protection Agency	T-RFLP	Terminal Restriction Fragment Length Polymorphism
Fe	Iron	TS	Total Solids
GC	Gas Chromatography	US	United States
GHG	Greenhouse Gas	USDA	United States Department of Agriculture
H ₂ S	Hydrogen Sulfide	VFA	Volatile Fatty Acid
HRT	Hydraulic Retention Time	VMP	Volumetric Methane Potential
I/S Ratio	Inoculum to Substrate Ratio	VS	Volatile Solids
ICP-MS	Inductively Coupled Plasma Mass Spectrometer	VSLR	Volatile Solids Loading Rate
K	Potassium	WA	Washington State
LCFA	Long Chain Fatty Acid	WSDA	Washington State Department of Agriculture
LTAD	Low Temperature Anaerobic Digestion	WSU	Washington State University
N	Nitrogen	WWTP	Wastewater Treatment Plant
N ₂	Nitrogen (gas)	XPS	X-ray Photoelectron Spectroscopy
Na	Sodium	Zn	Zinc
NH ₄ ⁺	Ammonium		
Ni	Nickel		
NPK ratio	Nitrogen Phosphorus Potassium		

Nutrient Recovery within Anaerobic Digestion and Bio-char Platforms

1.1 Nutrient Recovery for Anaerobically Digested Manure

Craig Frear and Quanbao Zhao

1.1.1 Introduction

One objective of the work carried out in partnership with the Washington State Department of Agriculture (WSDA) is to continue to advance the concept of integrating anaerobic digestion (AD) and nutrient management. This includes a major effort to continue commercialization of the patented Washington State University (WSU) nutrient recovery (NR) system. This objective fused outreach and extension with ongoing laboratory research focused on confirming and improving commercial-scale performance. Highlights include a draft fact sheet describing the rationale for AD and NR, which is summarized here. This fact sheet was instrumental in our extension efforts with the United States (U.S.) Environmental Protection Agency (EPA) Headquarters and Region 9 and 10 offices, who dealt with emerging dairy nutrient issues during this biennium. Major accomplishments also include the construction of commercial units utilizing the WSU technology at two digester facilities in Washington (WA) and two in the Midwest, with engineered plans for several other locations.

1.1.2 Background

1.1.2.1 Manure management and environmental issues in the United States

In the U.S., a considerable percentage of the cow inventory is characterized as being derived from concentrated animal feeding operations (CAFOs). In addition to producing milk, each dairy cow produces approximately 60 kg total nitrogen (N), 30 kg ammonia, and 7 kg phosphorus (P) as manure annually (ASAE, 2005). Unfortunately, this liquid manure is expensive to transport (Heathwaite et al., 2000). Therefore, dairy manure and its nutrient load are generally land applied to nearby fields, which can lead to excess applications of N and/or P. Nationally, in 2000, only 1% of large CAFOs (those with more than 1,000 animal units) were applying P at agronomic rates, while only 23% were applying N at agronomic rates (Mac Donald et al., 2009). A recent study of manure application to field corn, the receiving crop for more than half of all applied manure, confirmed that the vast majority of dairies applied to fewer acres than would be needed to meet best management practices for nutrient management (USDA-ERS, 2011).

Current manure management patterns contribute to a number of significant air and water quality concerns, including the following:

- *Phosphorus eutrophication:* Levels of soil P in localized areas throughout the U.S. have become critical, elevating concerns about runoff to waterways (Kiely, 1997). At typical

concentrations of 200–700 mg P/L, dairy wastewater runoff can easily cause total P levels in waterways to exceed 100 µg P/L, a level considered sufficient for provision of nutrient enrichment to biota (Bektas et al., 2004).

- *Nitrogen eutrophication:* Losses of N can also occur via soils runoff and leaching. These processes can occur at manure storage sites and field application sites. A considerable body of literature concludes that as N-load increases beyond plant assimilation needs, N losses to the environment accelerate (Dobermann, 2007; Ferguson et al., 1991; Schlegel et al., 1996; Vanotti & Bundy, 1994). Once lost from agricultural systems, N can migrate to lakes, rivers, estuaries, and coastal oceans. Overabundant nutrients can then lead to excessive growth of algae and aquatic weeds and subsequent oxygen (O₂) shortages (Carpenter et al., 1998), fish toxicity (Ward et al., 2005b), habitat loss (Jeppesen et al., 1998; NRC, 1993) and decreased species diversity (Sutton et al., 1993).
- *Ammonia volatilization and small diameter particulate matter:* On dairies and feedlots, an estimated 70% of total N in manure is lost as ammonia during manure management and application (CAST, 2002). In the U.S., it is estimated that as much as 25% of all gaseous ammonia emissions originate from ruminant animals (CAST, 2002). Ammonia is highly reactive and contributes to the development of small diameter particulate matter (PM 2.5) that has detrimental effects on overall air quality (Erisman & Schaap, 2004) and human/animal health (Archibeque et al., 2007; McCubbin et al., 2002).
- *Nitrate pollution:* Nitrates can also accumulate in the soil and migrate to the water supply where they can induce blue baby syndrome in infants under six months of age (Ward et al., 2005a).
- *Greenhouse gas emissions:* Although dairy CAFOs create direct and indirect emissions of greenhouse gas emissions (GHGs) throughout the production process (10–11 MT CO₂e/cow per year) (US-EPA, 2008; Oleson et al., 2006), a significant proportion of these emissions occurs during manure management.

1.1.2.2 Nutrient overloading issues in dairy regions of the western United States

Many of these water and air quality issues are acutely evident in Washington's Yakima Valley, which has high concentrations of dairies and cropland agriculture. Nutrient overloading has become a concern, and the U.S. EPA (2012) recently released its *Lower Yakima Valley Nitrate Study* that assessed likely sources of nitrate pollution (20% of wells > 10 mg/L)—including dairy farms and crop fields—in Lower Yakima Valley groundwater and drinking water wells. N eutrophication concerns are also present, particularly because the Middle Columbia River bull trout and the Middle Columbia steelhead (both listed under the Endangered Species Act) spawn or rear in this watershed. Separately, air quality monitoring results have indicated that the Yakima River Basin presently exceeds the 15 µg/m³ annual and/or 35 µg/m³ twenty-four hour PM 2.5 Standard (US-EPA, 2004).

Other dairy-intensive areas of the Pacific Northwest (PNW) and West are dealing with similar issues. Along the Canadian-U.S. border in Western WA, recent concerns over high nitrate levels in the regional Abbotsford-Sumas aquifer (Mitchell et al., 2005) have placed added pressure on dairies and other producers. Nitrogen-loading and nitrate issues have also received increased attention in dairy-intensive regions of California and New Mexico. In the Tulare Lake Basin and

Salinas Valley of California, Viers et al. (2012) estimated that the vast majority of nitrate loading occurred during the application of manure and inorganic fertilizer to fields. In this case, roughly a third of the N applied to crop fields was derived from dairy manure sources since dairy commodities are the top economic producers in the Tulare Lake Basin.

There are many factors that contribute to nutrient overloading, despite the negative environmental impacts:

- The expense of transporting manure to distant fields. This is particularly true for liquid manure, but also applies to “dry” manure, which contains significant moisture (Heathwaite et al., 2000; Henry & Seagraves, 1960; Ribaud et al., 2003).
- Reluctance to apply manure to food crops due to environmental and food safety concerns (Guan & Holley, 2003). This limits the land base available for manure application to forage fields (Mac Donald et al., 2009).
- Variability in the nutrient content and form in stored manure and the timing of nutrient availability to plants (especially for N). This can lead producers to apply extra manure and/or supplement with inorganic fertilizer (Alva et al., 2006; Davis & Dernoeden, 2002; Eghball et al., 2002; Power et al., 2001).
- The nitrogen-phosphorus-potassium (NPK) ratio of manure may not match the ratio needed by crops, necessitating additional inorganic fertilizer for proper nutrient balance (Frear et al., 2011b; Mac Donald et al., 2009).
- Broadcasting, a widely used and economical manure application method may encourage nutrient loss and runoff (Mac Donald et al., 2009).
- Crop producers’ tendency to target nutrient application toward high-yield goals, rather than average yields (USDA ERS 2009).

Manure is not necessarily bad, and in fact has a high potential value because of the nutrients it contains. Many crop producers use manure alongside organic fertilizers to improve NPK blend, application timing, or to reduce the economic risk associated with volatile spikes in fertilizer prices (USDA-ERS, 2011). However, nutrients in manure are only valuable when there is a nearby market for those nutrients - and meanwhile, dairy producers have to dispose of manure in a way that complies with stringent storage and application regulations that often specify loading rates and timing. This generates highly localized markets for manure with crop producers in some areas paying for manure while crop producers in other areas require dairies to pay them for accepting the manure. Manure management is thus a major consideration for dairy producers, with high potential costs in areas where there are few crop producers willing to accept manure (Mac Donald et al., 2009).

1.1.2.3 Recovering, concentrating, and partitioning nutrients from manures

Based on the increasing costs of nutrient management for dairy manure, increasing attention is being paid to the development of commercially viable NR technologies. Although no technologies are widely commercialized at present, several N and P recovery technologies have recently emerged that have the potential to improve nutrient management on dairies. Some of these technologies are most appropriately used on untreated dairy manure, while others are to be combined with AD as part of an AD system.

1.1.2.4 Benefits and challenges to nutrient recovery

NR technologies produce concentrated nutrient products that can be more economically transported than manure. In some cases, the NR processes also generate a product which is more stable, homogenous and predictable than manure. This can make the products more appealing to crop producers, who can store them, better control application rates, and in some cases control application method. In addition, blending of NR products with each other or with inorganic fertilizers has the potential to produce products with desired NPK balances and other characteristics. Lastly, processing time for these nutrient products and (in some cases) exposure to high temperatures can diminish real and perceived environmental and food safety risks that lead some crop growers to avoid manures. Some NR products, such as struvite and ammonium sulfate, are pathogen-inert chemicals.

However, in practice, most NR products are still in development. Products from various technological processes may be heterogeneous or have inconsistent form. They may also require further processing to dry or make product handling and application manageable. And products with diminished (but not eliminated) pathogen risks may still be unappealing to food crop producers.

In addition, markets for these products have not yet matured due to limited product availability and unproven fertilizer efficacy. Further development of dewatering technologies and consistency of fertilizer form, function, and performance are needed. Together, these steps could lead to market development and increased revenues adequate to provide cost-recovery for implementation of NR processes.

In addition to the nutrient products, NR processes leave a nutrient-poor wastewater that is less likely to exceed required nutrient regulations on nearby soils—a positive attribute. However, the wastewater that results from the AD/NR process is even more nutrient-dilute than it was prior to treatment, and can thus be more expensive to transport (on a per acre basis) if transport is still required for nutrient management. It is also difficult to apply precisely, and the NPK ratios are actually more unbalanced than manure, with much higher ratios of potassium (K) and other salts. Thus, it will be essential that cropland receiving the low-nutrient wastewater be effectively monitored for salt content. Changes in crop selection and rotation on dairy forage fields may be necessary to accommodate the distinctive characteristics of the nutrient-diluted wastewater.

1.1.2.5 The role of nutrient recovery in achieving environmental quality

Regulation has played an important role in CAFO nutrient management and undoubtedly will continue to do so. However, there are limits to the effectiveness of a purely regulatory approach. As Aillery and colleagues (2005) have pointed out, tighter regulation to protect water quality of N in manure applied to cropland has the potential to induce changes to manure management that reduce losses of N-nitrate by trading them for losses of N-ammonia (currently unregulated), with negative impacts to air quality. Implementing regulatory strategies for nutrient management without viable technology options to concentrate and export N from dairies will likely encourage further examples of this type of shifting.

Implementing NR technologies may be a cost-effective approach to improving nutrient management at a watershed level, through the replacement of imported chemical nutrients used by crop farms with manure-derived nutrients already in the watershed. However, it is important to note that nutrients can still be lost from NR products or from nutrient-diluted wastewater, especially if these are applied with improper application rates or timing. NR technology thus needs to be part of a comprehensive strategy at the watershed level to address issues of nutrient balance, equitable distribution of costs and benefits, and improved nutrient application timing and methodology.

1.1.2.6 Anaerobic digestion and nutrient recovery

On its own, AD is not a NR technology. The AD process creates an anaerobic environment (without O₂) in which naturally occurring microorganisms convert complex organic materials in manure and other wet organic wastes to biogas, a source of renewable energy (AgStar, 2010b). The process also reduces GHG emissions, decreases odors, stabilizes waste, and decreases pathogen counts (Martin & Roos, 2007; US-EPA, 2004). Although the process changes the form of N and P in manure, it does not appreciably decrease the total amount of nutrients, most of which are concentrated in the liquid effluent that is a product of the AD process (Frear et al., 2011b).

An increasing number of dairies that practice AD have begun to import and co-digest food processing wastes along with manure in order to enhance biogas production and profitability. However, this practice often exacerbates the existing nutrient management concern by increasing the import of nutrients to the dairy. In a study of co-digestion, Frear et al. (2011b) showed that supplementing manure with 16% non-dairy derived organic wastes by volume at a dairy in WA increased N and P by 57 and 23%, respectively.¹

AD also creates unique opportunities for NR. In addition to transforming nutrients from organic to inorganic forms, the AD process can assist in NR by providing important process inputs such as heat, electricity and processing infrastructure. It also alters the effluent's ammonia and solids concentration, temperature and form of P (Frear et al., 2011b). In return, the NR process can assist the AD process by generating a combined system that can lessen dairy producers' nutrient concerns—something AD alone simply cannot do. Furthermore, potential income from the sale of recovered nutrients contributes to the economic feasibility of an AD project. Galinato et al. (2013) showed that income from nutrient and fiber products can represent a substantial portion of a digester's gross revenue.

Nutrient recovery technologies also have the potential to stimulate adoption rates for AD. Adoption rates have been slow, with just over 181 digesters in operation on dairy farms in the U.S. as of May 2013, representing about 4% of dairy cows (www.epa.gov/agstar/projects). This number would need to increase considerably to meet the joint U.S.- dairy industry goal for the dairy industry to reduce its climate impact by 25% by the year 2020 (ICUSD, 2011). Integrated NR technologies have the potential to address one of producers' top concerns related to AD adoption, and thus may be more appealing than stand-alone AD technologies. As one dairy farmer in the

¹ Co-digestion also produced a 110% increase in biogas and a tripling of gross revenues from anaerobic digestion, with 72% of all gross revenues directly attributable to outside organics digestion.

PNW stated, “We don’t necessarily want to be energy producers. We want to milk cows. But...if an anaerobic digester can help us solve our [nutrient management-related] manure problems, producing energy and mitigating GHG emissions will be welcome side benefits.”

A combined AD-NR system has greater capital and operating costs, but also (depending on the system) has the potential to generate greater revenues and profits. This “add-on” NR technology reflects an ongoing trend to use AD technologies as a “platform” for other technologies that work synergistically to provide operational and economic benefits. Renewable natural gas (RNG) is probably the most developed of these add-on technologies, and has been particularly important for improving project economics in regions with low electricity prices.

1.1.3 Commercialization Progress and Performance

As noted, four facilities (3 dairy and 1 poultry) now have commercial WSU NR systems working in concert with AD units. Under funding from this Appendix A and United States Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS), key deliverables included fact sheets summarizing the techno-economic and performance capabilities as well as a field day in July 2013 to showcase both the fact sheets and the system. Due to construction delays and ongoing process improvements, these fact sheets are only now being developed and, unfortunately, performance goals (70% ammonia recovery) have not yet been realized. Current operation remains at 40–50% ammonia recovery. However, ongoing engineering and science efforts are aimed at improving the performance and reducing costs. Future fact sheets will hopefully be able to document achievement of techno-economic goals and identify key determinants of positive economics, including scale and pricing. Submission of fact sheets is planned for the end of June with the field day scheduled for July 10.

Specific accomplishments this biennium include:

- Construction and operation of two facilities in Washington: FPE Renewables in Lynden and Rainier Biogas in Enumclaw (as well as completion of a third dairy facility in Chilton, Wisconsin, in late spring 2013).
- While performance at both Washington facilities is still on the order of 40–50% ammonia recovery, insufficient hydraulic retention time (HRT) has been identified as a key cause. HRT is being increased through additional operating capacity (Lynden) and/or attempting to maintain design flow (Enumclaw).
- Engineering solutions have been developed to address additional technical hurdles related to foaming, modulating flow, noise reduction, acid pH control, cold climate operation, etc.
- Numerous difficulties in operating NR alongside co-digestion have been discovered, with co-digestion, in general, resulting in less complete digestion. This leads to an effluent that contains organics and has a lower pH, making NR more difficult. While it has been difficult to solve this issue, it does show the fine linkages between AD and NR. In particular, effective and complete AD is an important precursor for effective NR.
- Discussions, testing and projects made possible by awarded grants continue to make progress towards developing the markets for NR products. This process is still ongoing, and somewhat complicates the production of techno-economic fact sheets.

- Numerous linkages between dairies, regulators, the dairy industry and project developers have been cultivated. If system performance can be improved and capital and operating costs reduced, these relationships will be important to future adoption of NR technologies.
- Integration of the final biogas scrubbing/pH return step was proved at lab-scale, pilot-scale and now at commercial-scale, allowing for near 100% scrubbing of hydrogen sulfide (H₂S) from biogas and return of effluent pH to near neutral.
- Designs for a large 8,000–12,000-cow AD facility with complete NR are moving forward in Outlook, WA, thanks to the considerable outreach/extension efforts, along with the data available from the commercial NR facilities currently in operation.

1.2 Nutrient Recovery within a Bio-char Platform

Mathew Smith, Waled Suliman and Manuel Garcia-Perez

1.2.1 Introduction

Nitrogen and P are in relatively high concentrations within dairy manure. As discussed in the previous section, the N and P normally is stored with manure in lagoons and then is land-applied. Application in excess of the amounts required by crops can result in runoff of excess N and P and leaching of nutrients to groundwater, posing an environmental risk. This project studied the efficacy of inexpensive bio-chars to remove N and P from anaerobic digester effluents and increase soil cation/anion exchange capacity.

1.2.2 Background

Because bio-chars are significantly more stable than the fast- and slow-cycling fractions of soil organic matter, the effects of bio-char additions to soil can have significant long-term benefits on soil fertility and carbon (C) sequestration (Lehmann, 2007; Lehmann et al., 2006). It is also known that acidic functional groups on the surface of bio-char can significantly increase N adsorption capacity. These functional groups can be generated with oxidizing agents such as steam and carbon dioxide (CO₂) at high temperatures (300–700°C) or with ozone at room temperature (Lehmann et al., 2011).

The adsorption of P in the form of phosphate depends on the cations found in the ash part of bio-char (Agyei et al., 2000; Agyei et al., 2002; Lu et al., 2009; Namasivayam & Sangeetha, 2004; Oguz, 2005; Xue et al., 2009). The addition of appropriate metal ions to the structure of the biomass should aid in creating additional basic sites on the char surface, which will become positively charged in solution and attract anions to the surface.

If bio-char produced from the pyrolysis of digested fibers and woody biomass can be economically activated to enhance its capacity to retain nutrients (N and P) from digester effluent, then a bio-char platform could help reduce the environmental footprint of dairy operations. Figure 1.1 shows a scheme of the integrated system proposed with the streams numbered.

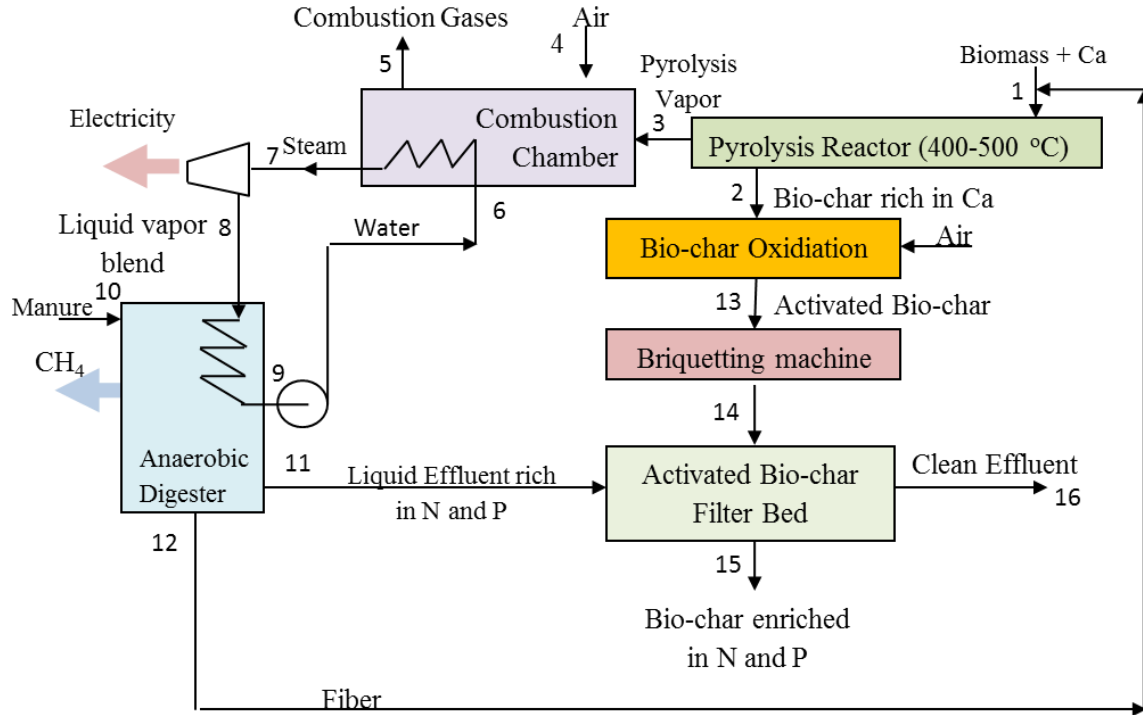


Figure 1.1: Integrated pyrolysis-AD systems for the production of methane, heat, and bio-char enriched in nutrients and clean water

1.2.3 Methods and Results

1.2.3.1 Evaluation of bio-chars with high surface area, calcium oxide, and iron sulphate/oxide for phosphate removal

Preparation: AD fiber was first dried at 105°C for 24 hrs and ground. Then, 100 g of ground sample was acid-washed in a 2% nitric acid solution to remove metal impurities. The sample was mixed with 1 L of solution comprising 30 mL of 70% nitric acid and 18 Mohm purified water and stirred for 2 hrs. A series of preliminary calcium (Ca) and iron (Fe) additions were carried out following the scheme outlined in Figure 1.2.

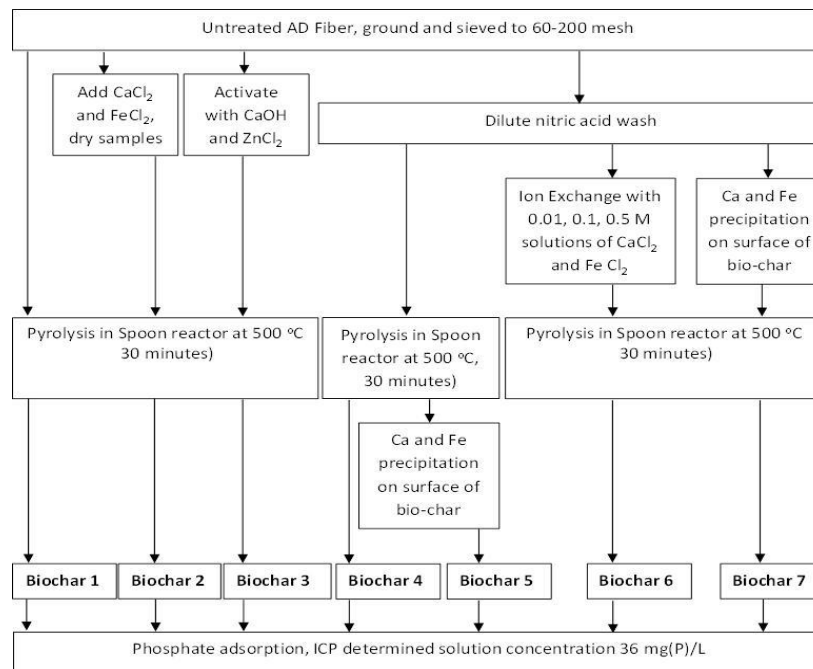


Figure 1.2: Diagram of treatments to screen pre- and post-pyrolysis treatment of bio-chars

Analyses: Ash quantification was performed with a high pressure digestion, elemental analyzer, and inductively coupled plasma mass spectroscopy (ICP-MS); char characterization was studied by scanning electron microscopy (SEM); P adsorption was analyzed by ICP-MS. Increasing the concentration of Ca and Fe was found to have only mild effects on the concentrations of most cations.

Conclusions: Ca precipitates were by far the most effective method for increasing the phosphate retention of bio-char. Other methods resulted in either little effect (Fe samples) or heavy precipitates when tested without heavy washing (calcium hydroxide and zinc chloride). The best results for adsorption were obtained with pre-pyrolysis precipitation of Ca on the bio-char surface. This treatment resulted in 53% removal of phosphate after 12 hrs. Results of all tests are shown in Table 1.1.

Table 1.1: Summary of P removal and mineral leaching from various treated chars

Sample	Fe leached mg/L	Ca leached mg/L	Total leaching mg/L	P-removal After 12 hours
Biochar 1	2.90E-04	4.40E-04	0.028	11%
Biochar 2 Fe Dry	0	0.07	0.09	7%
Biochar 3 Ca(OH) ₂ activated	Inconclusive, heavy precipitation in solution			
Biochar 3 ZnCl ₂ activated				
Biochar 4	0	0.0002	0.001	7%
Bio-Char 5 Ca Precipitate	0	0.37	0.45	98% (filtered) 35% un-filtered
Bio-char 5 Fe Precipitate	0	0.15	0.15	11%
Biochar 6 Ca 0.5M	0.0001	0.0005	0.011	3%
Biochar 6 Fe 0.5M	1.30E-04	0	0.011	0%
Bio-char 7 Ca Precipitate	0	0.023	0.071	53%

1.2.3.2 Evaluation of the potential of low temperature oxidation to generate acidic functional groups on bio-chars

The purpose of this study was to examine the effect of pyrolysis temperature on O_2 complexes formed on bio-char surfaces by a post-pyrolysis air oxidation at 250°C . Bio-char samples were produced under six different temperature regimes and then oxidized via air at 250°C for 30 mins. The O_2 functional groups were investigated by x-ray photoelectron spectroscopy (XPS). Results showed the formation of various oxygenated functional groups (carbonyl, carboxyl and hydroxyl) on the surface of oxidized bio-chars.

Preparation: Two dairy fiber samples were collected. Each sample was ground and three independent oxidation methods were tested including cold plasma, ozone, and O_2 at $200\text{--}350^\circ\text{C}$. Two different woody feedstocks (hybrid poplar and pine) were initially air-dried and milled to $590\ \mu\text{m}$. Thereafter, the samples were slow-pyrolyzed at varying temperatures ($350, 400, 450, 500, 550$ and 600°C). Charring time was 30 mins, and charring batches consisted of $< 1\ \text{g}$ of air-dried, ground biomass. Nitrogen gas (N_2) was used as a purge gas. Half of the produced bio-chars were then exposed to air at 250°C in order to oxidize and add oxygenated functional groups to the surfaces. The effect of each oxidation on the total carboxylic acid groups present on the char surface is given in Figure 1.3.

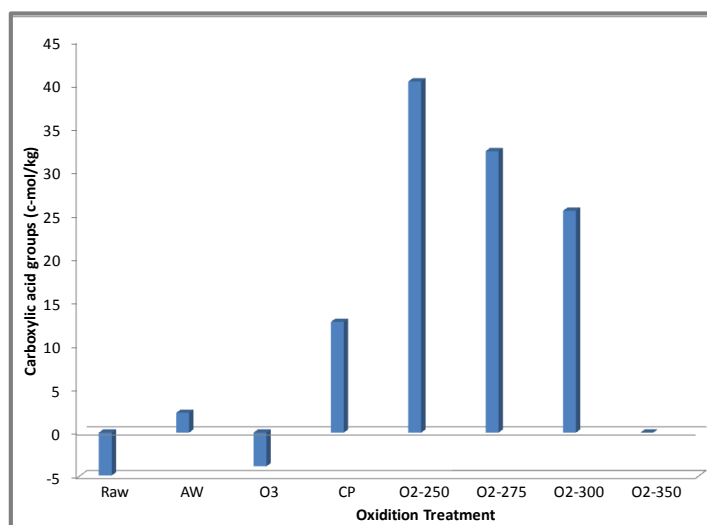


Figure 1.3: Formation of carboxylic acid groups under different oxidation conditions

Conclusions: The best results were obtained by air oxidation, with 250°C being the most effective temperature tested (Figure 1.3). At increasing temperatures, lower acid concentrations were detected due to the poor thermal stability of these groups. For example, at 350°C these groups were no longer stable on the surface and no longer detected by titration. The value in determining carboxylic acid groups from the previous results showed a direct, linear correlation between the quantity of acid groups detected by titration and the removal of ammonium (NH_4^+) from a single component system, as shown in Figure 1.4.

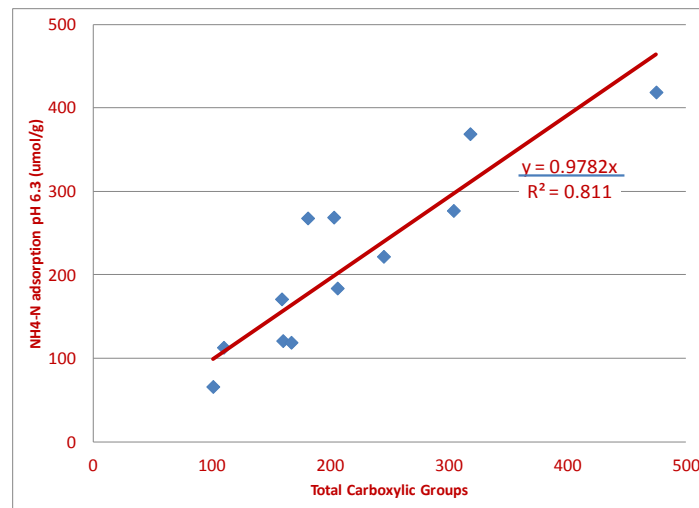


Figure 1.4: Ammonium adsorption vs. total carboxylic groups from ozone oxidation

1.2.3.3 Evaluation of nitrogen and phosphate removal in waste streams from anaerobic digesters

Based on the ionic phosphate retention results obtained using model phosphate solutions, Table 1.2 was created to show the annual tons of char required to remove phosphate at a given concentration from the effluent water produced by a hypothetical 1,000-cow dairy. These results are based on the assumption that each cow produces 32 gallons of effluent per day. These results indicate that approximately 1–5 tons of char per day would be required at a concentration of 50–100 mg (P)/L. This concentration assumes that 80–90% of P exists as colloidal solids that can be removed by other methods such as filtration or gravity settling. If all of the P is assumed to be ionic phosphate, 10–20 tons of char per day would be required to treat a 500 mg (P)/L waste stream.

Table 1.2: Annual tons of char required to remove ionic phosphorous from the effluent water of 1,000-cow dairy given different retention rates

Ionic Phosphorous Concentration (mg/L)	Annual tons of char for 1000 cow AD system		
	Phosphate Retention on Char		
	10 kg/ton	15 kg/ton	20 kg/ton
1	13	9	7
10	133	89	67
20	267	178	133
50	667	444	333
100	1333	889	667
150	2000	1333	1000
200	2666	1777	1333
500	6665	4444	3333

Typical results from oxidation of AD char, hybrid polar char and pine wood char showed approximately 30–40 centimoles NH_4^+ /kg char to be a reasonable upper estimate of the potential cation exchange capacity and retention capacity of chars given ideal conditions. This translates to approximately 6–7kg NH_4^+ /ton of char as the upper retention limit. Table 1.3 was created to

estimate the annual tons of modified char required to treat dairy effluent at various total ammonia nitrogen (TAN) loadings at a 1,000-cow dairy operation. The upper limit of TAN loading is 1.5 g/L, with 200–500 mg/L considered as the probable range after NR. The results indicate that 5–40 tons per day of oxidized char would be required to treat the effluent produced. At the lower range, this is potentially a reasonable quantity; however, the upper range may generate significant material handling difficulty. An estimated maximum daily production of 115 tons is required to treat a 1.5 g/L effluent stream assuming 2 kg NH_4^+ /ton retention.

Table 1.3: Annual char required to remove TAN from solution in a 1,000-cow dairy (*left*) and metal cation concentrations in digester effluent based on dairy manure (*right*)

TAN (mg/L)	Annual tons of char for 1000 cow AD system			Dairy Digester Effluent			
	Ammonium Retention on Char			Species	Conc (g/L)	species	Conc (mg/L)
	2 kg/ton	4 kg/ton	6 kg/ton				
10	280	140	93	Na	1.2	Fe	72
50	1398	699	466	Mg	0.4	Al	25
100	2796	1398	932	K	10.0	Mn	24
200	5593	2796	1864	Ca	2.9	Cu	11
500	13982	6991	4661			Zn	13
1000	27964	13982	9321			Ba	2
1500	41946	20973	13982				

An additional concern present for NH_4^+ recovery is competitive inhibition by other cationic species present in the effluent. An example of cation concentrations in a dairy effluent is given in Table 1.3. Unfortunately, NH_4^+ absorption has been shown to suffer considerably due to the competitive inhibition by sodium (Na) and K, both of which are present in significant quantities in the solution. This competitive inhibition means larger quantities of char will be required to handle NH_4^+ present in the effluent. On the other hand, bio-char could be a means for simultaneously reducing K in the effluent.

Based on the above results, effective NH_4^+ filtration may be feasible if ideal conditions for the generation of high surface area and high acidity chars can be achieved at pyrolysis temperatures between 550 and 600°C and oxidation for less than 1 hr at a temperature of approximately 250°C. Any filtration system developed will depend heavily on the final concentration of TAN requiring filtration and the concentration of competing cations such as Na and K that are present in the effluent.

1.2.3.4 Design model for a portable packed bed adsorption column for phosphorus removal

Based on the results obtained from experimental studies, researchers examined the feasibility of a filter that could be deployed via a tractor trailer to smaller dairies to pump and filter lagoon water. The concept is shown in Figure 1.5. To be feasible, the filter should be able to filter at least a month's worth of lagoon water in under 8 hrs (1 day's work) with removal of at least 75% P. The feasibility of this goal was tested using a hypothetical scenario of a 100-cow dairy, producing 12 m³ of waste water containing 1.5 kg/m³ phosphate. Under these conditions, 7.33 tons of char would be required to treat effluent accumulated over a one month period; details can be seen in Figure 1.6 (left). For the filter model, the minimum char requirement was tripled to ensure adequate

removal. The removal efficiency of this filter was modeled assuming ionic phosphate concentrations of 0.025–1.5 kg/m³.

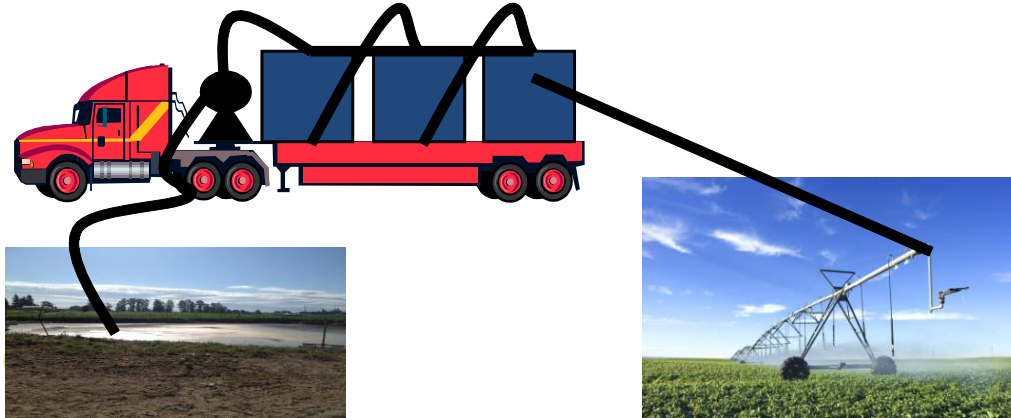


Figure 1.5: Portable lagoon filtering system

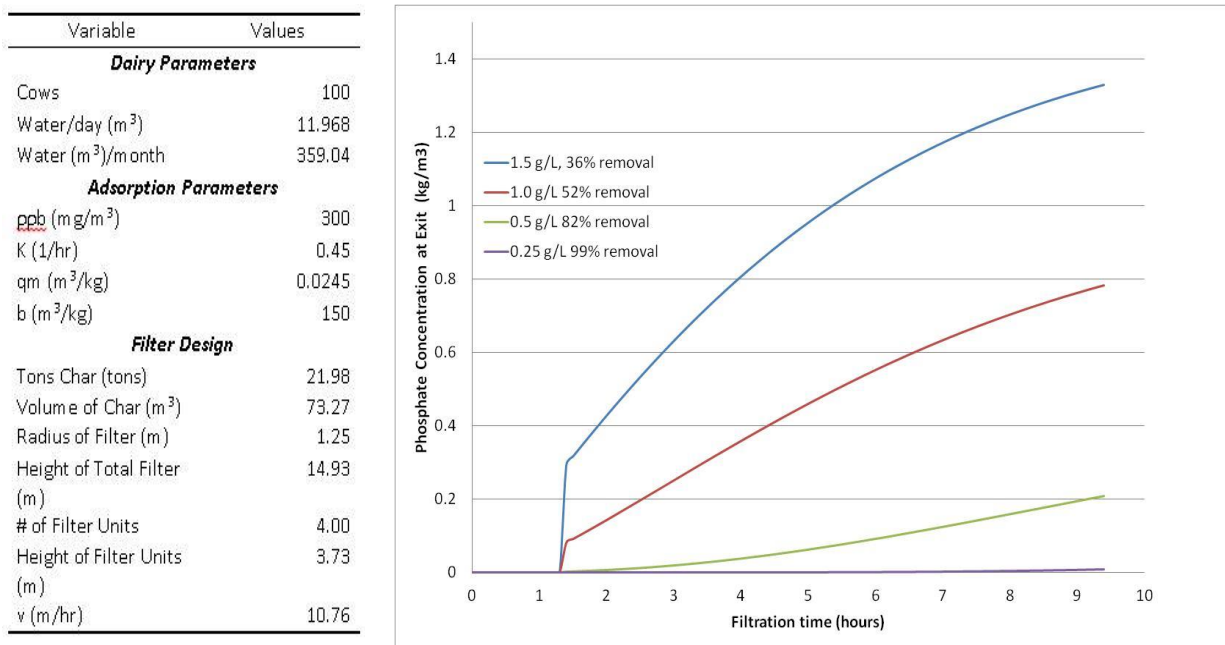


Figure 1.6: Char requirements (*left*) and concentration profile for adsorption with Ca-infused bio-char in a packed bed column (*right*)

Using the adsorption data for the bio-char with Ca precipitation at pH 12 (data not shown), the 8-hr solution concentration profile was generated (Figure 1.6, right). A residence time of 1.4 hours is required to pump the effluent water in 8 hrs. The list of variables and outputs are given in Figure 1.6. From these results it is apparent that total removal of phosphate is not possible at a 1.5 kg/m³ concentration. To achieve total reduction of 75% or more of phosphate, a maximum concentration of approximately 0.5 kg/m³ is required. At an initial concentration of 1.5 kg/m³, filtration of 360

m³ of water would result in total phosphate reduction of approximately 36%. This translates to removal of 198 kg PO₄ from the effluent. At this level, the market value of phosphate is insufficient to support the cost of filtration. Thus, additional economic or regulatory incentives will be required to advance this concept.

1.2.4 Conclusions

Using the data collected in previous experiments, researchers determined the approximate amount of P that could be removed from liquid wastes on a hypothetical 1,000-cow dairy operation using a bio-char filtration system deployed via a truck trailer. Results indicated that approximately 1 to 5 tons of char per day would be required to remove P from dairy manure effluent. If all the phosphorus is assumed to be ionic phosphate, approximately 10 to 20 tons of char per day would be required.

Similar estimations were made on the amount of modified char required to treat dairy effluent at various TAN loadings at a hypothetical 1,000-cow dairy operation, assuming an upper limit of 1.5 g/L ammonia in effluent, with 200–500 mg/L NR. Results indicated that 5 to 40 tons per day of oxidized bio-char would be required to treat the effluent produced, with a maximum of 115 tons. Though the lower range is reasonable, the upper range would likely lead to significant material handling difficulty and increase the cost of the process beyond what is deemed viable. An additional concern is competitive inhibition of NH₄⁺ recovery by Na and K, both of which are present in significant quantities.

Based on these results, additional work needs to be done before this is a viable technology at dairy operations utilizing AD as a waste management strategy. Future work should focus on achieving ideal conditions for the generation of high surface area and high acidity chars at pyrolysis temperatures between 550 and 600°C and oxidation for less than one hr at a temperature of approximately 250°C. In addition, any filtration system developed will depend heavily on the final concentration of TAN requiring filtration and the concentration of competing cations such as sodium and potassium present in the effluent. Nonetheless, these studies provide concrete evidence that nutrients (N and P) can be removed from AD effluent, thus providing one more way for AD and pyrolysis to complement each other to mitigate environmental problems at CAFOs.

Flush Dairy and Anaerobic Sequence Batch Reactor Approach

Jingwei Ma, Liang Yu, Quanbao Zhao, Craig Frear and Shulin Chen

2.1 Introduction

There are three general systems used for collecting and storing manure at dairy operations: dry, scrape, and flush (see Figure 2.1). Of the three, flush systems, which use copious amounts of recycled wastewater to dilute the manure for ease of transport through and around the farm (Frear et al., 2011a), are commonly the preferred strategy. This is because flush systems reduce labor and mechanical failure compared to dry and scrape systems. In particular, flush systems have become increasingly popular on large dairy operations that need to move manure long distances between barns. Since economics dictate that most digesters operate on large dairies, flushed manure is a common feedstock at dairy AD operations.



Figure 2.1: Different manure management strategies available for dairy operations

Unfortunately, flush systems often require much larger downstream treatment systems because of the enormous volume of water they use. When flushing is coupled with AD, the size and heating requirement for the digester are vastly increased compared to scrape system, which directly affects the economics of the AD operation. As a result, farms wanting to implement AD have been either forced to switch to a scrape system or utilize modified or new digester technologies.

The most common commercially applied modification separates the liquid and solid phase, spinning off the supernatant liquid and thickening the solid sludge fraction. This concentrates the manure to 6–10% total solids (TS) and reduces the volume of manure needing treatment. The

concentrated manure is then most commonly treated in a plug-flow digester while the liquid portion goes back to the flush tower system, untreated, for reuse on the farm.

Research has shown that the removal of the liquid phase before digestion can reduce methane (CH_4) production by roughly 50%, since biogas generating compounds are equally distributed between the solid and liquid phases of the flushed dairy manure (Frear et al., 2011a). This reduction in CH_4 capture can negatively impact GHG mitigation efforts because a large portion of the organic material will decompose naturally, releasing CH_4 .

A second approach has been studied and has seen commercial application at a small dairy located at the University of Florida using high rate reactors with supporting media to retain solids (Wilkie et al., 2004). This approach separates out the solids and treats only the liquid portion of the flushed manure in a fixed-film, high rate reactor. Artificial supporting media is used in this type of reactor to promote microbial growth in the form of bio-films with the goal of enhancing the efficiency of the reactor to handle the high volume of liquid. As with the previous technique, CH_4 production is reduced because some of the waste (in this case the solid phase) goes untreated. In addition, potential clogging of the supporting media requires a strict screening process prior to digestion, impacting the viability of the process (Wilkie et al., 2004).

The significant drawbacks of both existing systems mean that technical innovations in digester design could still improve the capture of the full CH_4 potential of the flushed dairy manure, and therefore the economics of AD for flush systems.

2.2 Methods and Results

Since the fibrous solids contained in dairy manure have been shown to act as effective natural biomass carriers for high rate and simultaneous methanation of both solid and liquid phases (Frear et al., 2011a), the use of natural bio-films to enhance the biogas potential was investigated at WSU. Figure 2.2 illustrates the presence of anaerobic microbes attached to the fiber contained in flushed dairy manure. The high affinity of microbes to dairy manure fibrous solids could replace the need for costly supporting media in AD and lead to effective approaches for treating both the solid and liquid phases.

Building upon previous research, an anaerobic sequencing batch reactor (ASBR) was developed to retain the active biomass through gravity settling and selection pressure. The fiber, known to harbor anaerobic microorganisms, was used as a supporting system to increase biomass retention in the reactor. Figure 2.3 depicts the ASBR process. The study revealed bipolar effects on active biomass retention in SBR; both short and long settling times were able to retain high concentrations of active microbes (Wang et al., 2011). The results from this investigation led to a novel bacterial retention technology that could reduce the cost, maintenance and clogging issues currently hindering the adaptation of artificial support media. It also has the potential to increase digester efficiency.

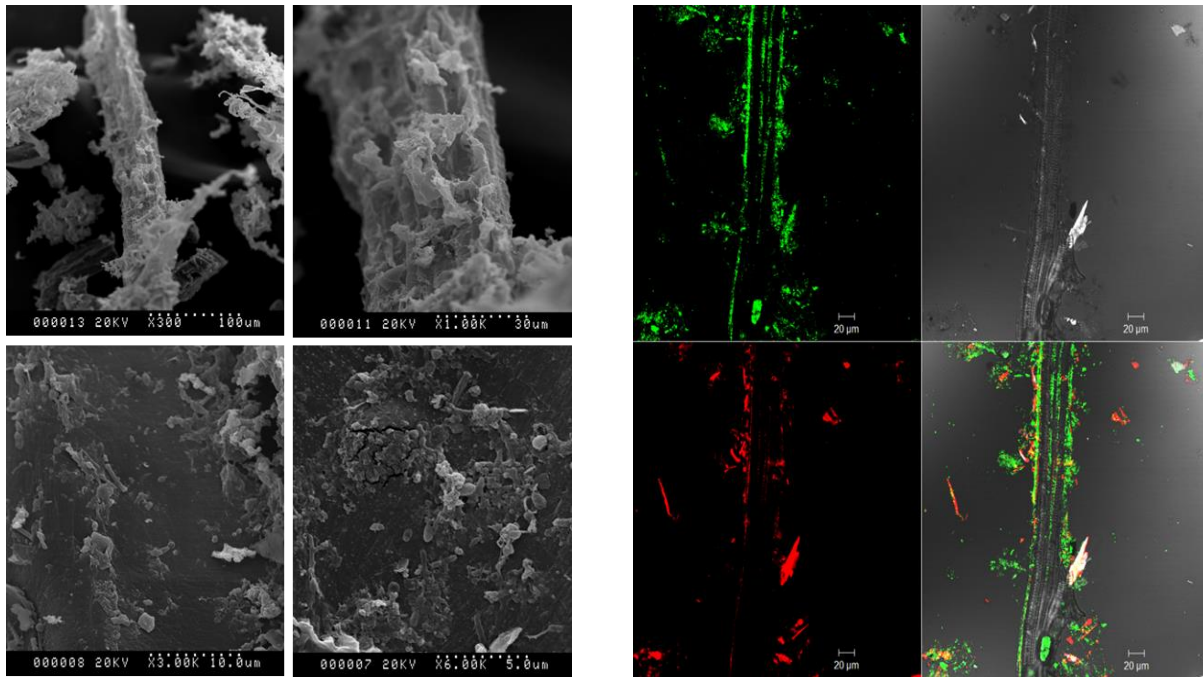


Figure 2.2: Scanning electron microscope rod and coccoid microbial cells attached to the fiber (left) and fluorescence *in situ* hybridization image shows fermentative bacteria attached to the surface of fiber and methanogens distributed on the outer layer of bio-biofilm (right)

Thereafter, the use of fibrous material as a natural biomass carrier was investigated in the ASBR at psychrophilic temperature (22°C), since other research has shown that an effective energy balance for such dilute waste streams is most attainable at lower temperatures (Wilkie et al., 2004). This could potentially allow digesters to be deployed in agricultural settings that experience more dramatic temperature changes (e.g. northern latitudes). Results from this research showed that an ASBR not only improved CH₄ production but also reduced the necessary HRT and temperature required to achieve a similar treating efficiency when compared with current technologies (Ma et al., 2013). A kinetic model that was developed indicated the best HRT and organic loading rate (OLR) were 4–6 days and 0.5–1.3 g volatile solids (VS)_{loaded}/L/day, respectively.

After the optimal HRT and OLR were achieved, an investigation was performed to identify the species of methanogens dominating the digestion process at the low HRT and temperature. This is important because a high level of methanogen activity is necessary to maintain an efficient AD process and avoid the accumulation of volatile fatty acids (VFA) that could cause inhibition and digester failure. Through analysis using 16S rRNA and terminal restriction fragment length polymorphism (T-FRLP), the ASBR operating at short HRTs was shown to be capable of establishing a *Methanosarcina*-predominated population. This is notable because *Methanosarcina* can lead to a more stable and efficient anaerobic process. *Methanosarcina*, the most versatile methanogens, have a higher substrate utilization rate, growth rate and cell yield when exposed to an environment with relatively high acetate and hydrogen concentration, such as is experienced in dilute manure treated at short HRT. This allows them to handle environmental changes that other species (i.e. *Methanosaeta*) cannot. Figure 2.4 illustrates the cluster of assigned operational

taxonomic units (OTUs), which indicated that *Methanosarcina* was the dominant CH_4 producing *Archaea* in the ASBR digester.

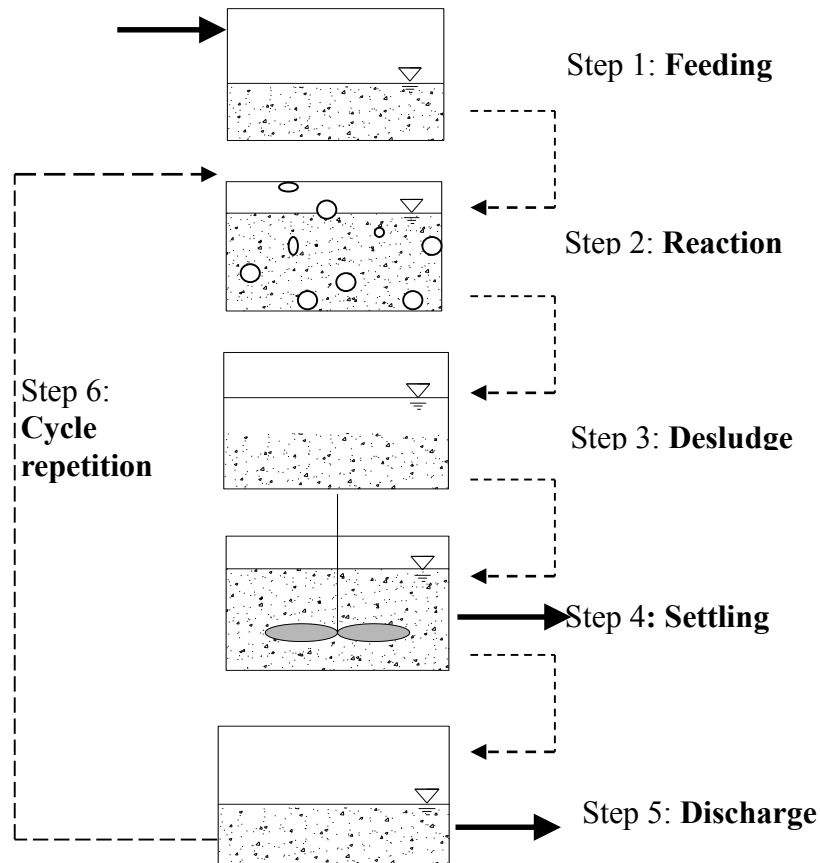


Figure 2.3: ASBR process divided into six stages to enhance the retention of biomass for improved biogas production and digester efficiency (Wang et al., 2011)

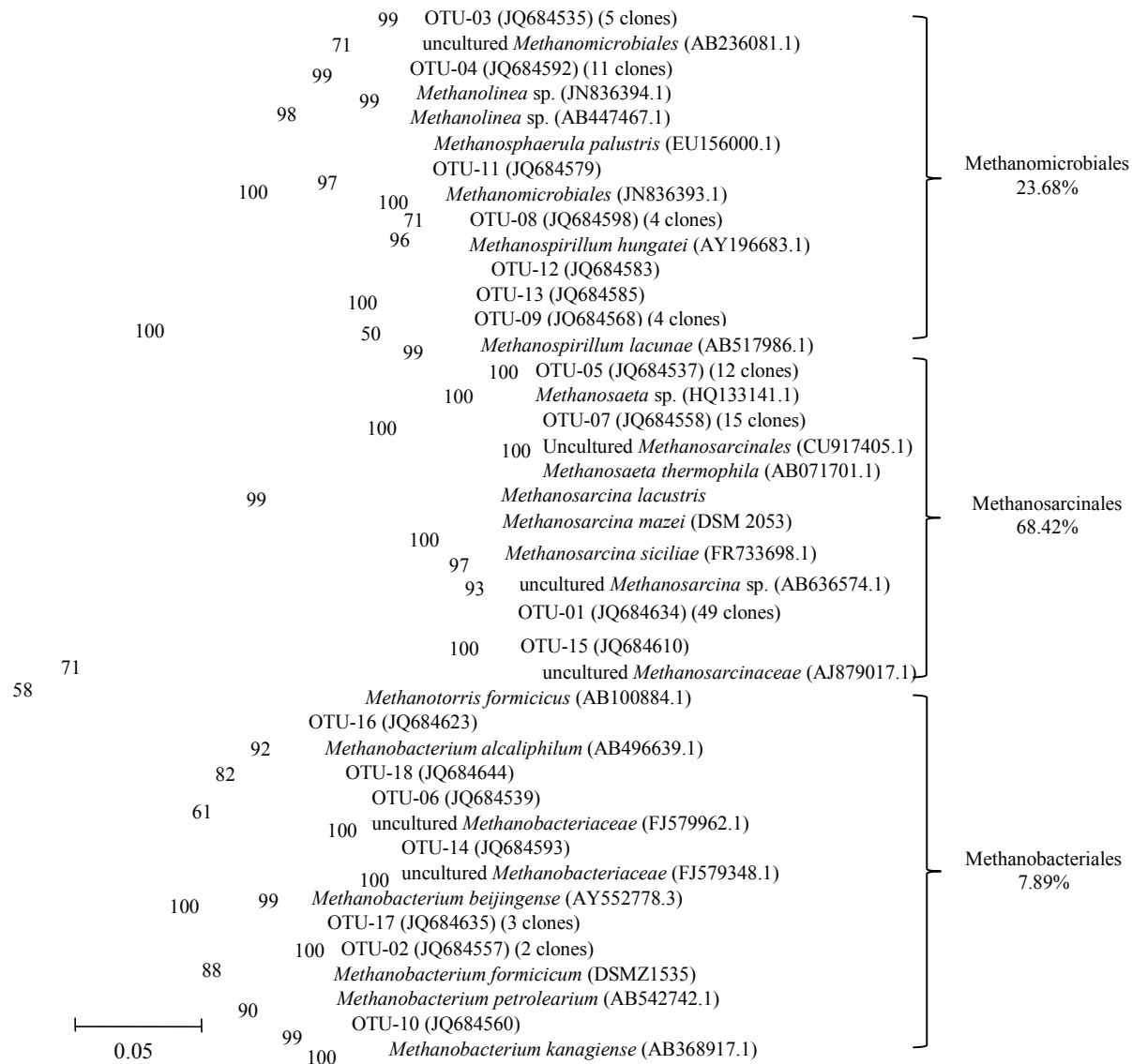


Figure 2.4: Phylogenetic relationship among 18 OTUs with partial 16S rRNA gene sequences of known methanogenic *Archaea* based on the neighbor-joining analysis

2.3 Conclusions

This study provided insight into how the anaerobic community shifts in response to operational parameters. At the optimal HRT of 4 to 6 days and OLR of 0.5–1.3 g VS_{loaded}/L/day in an ASBR reactor at a temperature of 22°C, analysis using 16S rRNA and T-FRLP indicated that the ASBR had established a population dominated by *Methanosarcina*. Beyond this specific study, the techniques utilized here to investigate anaerobic community shifts should provide a basis for identifying pathways for improved biogas and co-product production in the future.

Biogas Purification within the Anaerobic Digestion/Nutrient Recovery Platform

Nicholas Kennedy, Quanbao Zhao, Craig Frear and Shulin Chen

3.1 Introduction

Biogas derived from the AD of dairy manure consists of CH₄ (55–70%) and contaminants including CO₂ (30–45%), low concentrations of H₂S (300–4,500 ppm), and trace levels of halogenated hydrocarbons, N₂, O₂, organic silicon and water vapor (Liebrand & Ling, 2009). The three main contaminants – CO₂, H₂S and water vapor – can lead to problems when utilizing biogas as a renewable source of energy such as RNG (de Hullu et al., 2008), even though these impurities are lower in concentration in dairy manure biogas than in biogas produced from sewage sludge or at landfills.

Of these impurities, CO₂ is not detrimental to equipment or human health, but does decrease the energy potential of biogas because it is inert. On the other hand, H₂S, which is produced by the breakdown of proteins and other sulfur-containing compounds during hydrolysis, is detrimental to an internal combustion engine as well as to the environment and human health. Even at low concentrations, H₂S has an unpleasant odor and can be life threatening (Speece, 1996). Furthermore, this contaminant is highly undesirable in energy-recovery processes because it converts to unhealthy and environmentally hazardous sulfur dioxide and sulfuric acid (Abatzoglou & Boivin, 2009). Unless they are removed, these H₂S-derived contaminants limit the use of biogas to on-site combined heat and power (CHP), whereas many economic assessments have suggested that a higher value use is possible and beneficial for AD projects (Coppedge et al., 2012; Liebrand & Ling, 2009; Murphy et al., 2004). Figure 3.1 describes some of the different pathways for biogas processing and use. Higher profit potential is achievable when the biogas is upgraded to pipeline quality natural gas or compressed natural gas also known as RNG.

Due to the presence of unwanted compounds, many techniques that were originally developed in the natural gas and oil industries to remove contaminants have been adopted for biogas upgrading: water scrubbing, chemical absorbents, pressure swing adsorption, and cryogenic absorption, among others (Kapdi et al., 2005). Unfortunately, all these techniques require complicated and often expensive infrastructure to achieve the amount of cleaning required for injection into natural gas pipelines and transportation vehicles (de Hullu et al., 2008). This has severely hampered the adoption of biogas upgrading on dairy farms in the U.S. EPA AgSTAR, a voluntary outreach and educational program promoting the recovery and use of CH₄ from dairy anaerobic digestion, estimates that, of the 181 dairy based digester projects in the U.S. in 2013, one is currently upgrading biogas to pipeline quality gas and four are upgrading biogas to CNG vehicle fuel. To facilitate biogas upgrading on dairy farms and other organic waste facilities, cheaper techniques are needed.

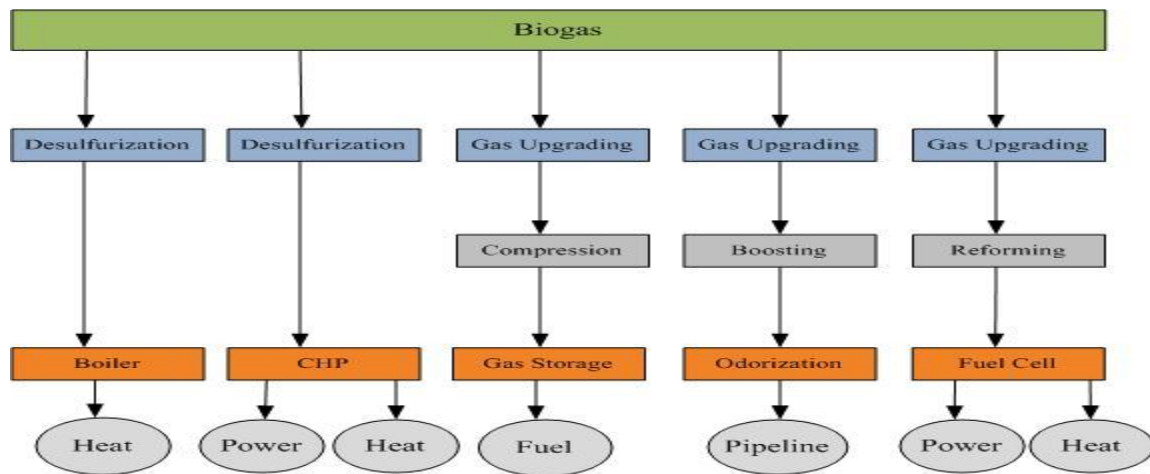


Figure 3.1: Multiple paths through which biogas can be used as renewable energy/fuel (Weiland, 2006)

One technique that could be used to remove both H_2S and CO_2 is to use the alkaline effluent that is produced as a byproduct of the combined AD and ammonia recovery process developed at WSU (Zhao et al., 2012). This alkaline effluent can be used as an inexpensive, on-site absorbent while at the same time returning the AD effluent pH back to near neutral $\text{pH} < 7.5$ in preparation for land application (Zhao et al., 2012). Figure 3.2 depicts the integration of the AD and ammonia recovery process with the proposed H_2S and CO_2 removal process.

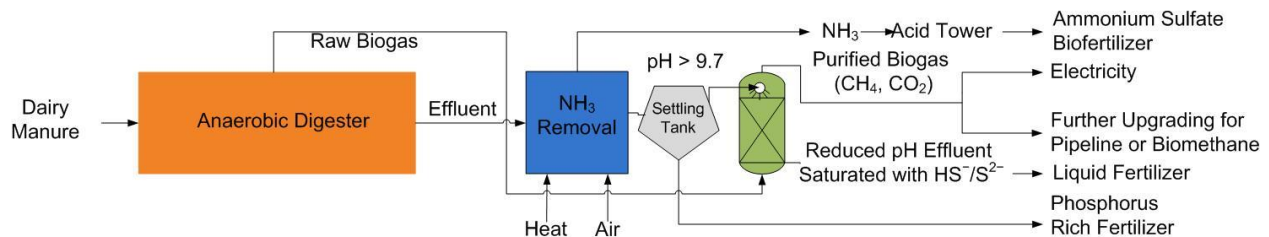


Figure 3.2: Ammonia and P recovery system with attached H_2S and CO_2 removal

Research into the selective removal of H_2S over CO_2 was conducted using a bubble column reactor to treat biogas using effluent from the AD and ammonia recovery processes. Because the biogas-to-effluent ratio of the combined system (AD/NR) is fixed at around 20:1 (biogas-to-liquid), a major goal of this study was to determine whether 100% removal of H_2S was possible at this given ratio. Selectively removing H_2S over CO_2 was investigated because H_2S is the most problematic impurity.

3.2 Methods and Results

The first experiments were conducted to see if the selective removal of H_2S over CO_2 from biogas was possible using the alkaline effluent as the absorbing media. The solubility of H_2S is only about three times greater than that of CO_2 in water. With such similar physical properties, mass transfer becomes a particularly important factor to selectively remove H_2S over CO_2 . In the majority of

applications where a bubble column is utilized, the gas-side mass transfer can be regarded as negligible (Kantarci et al., 2005). Unfortunately, H_2S has been found to have a dominant gas-side resistance to mass transfer (Bendall et al., 1983; Garner et al., 1958; Srinivasan & Aiken, 1988; Yih & Sun, 1987). This is why most selective H_2S purification is performed in an absorption apparatus that favors a gas-film controlled process (e.g. packed bed, spray tower) (Wallin & Olausson, 1993). However, CO_2 has dominant resistance to mass transfer on the liquid-side, a feature that the authors think can be exploited in a bubble column. This resistance can be attributed to CO_2 having a slow initial reaction in solution, unlike H_2S .

Bubble columns have many advantages over other absorption apparatuses including excellent mixing and mass transfer characteristics, lack of moving parts, easy temperature control, little maintenance, elimination of clogging issues, and low costs of construction and operation (Kantarci et al., 2005). Disadvantages include an inability to easily control the gas-film, short gas-phase residence time, back mixing in both the gas and liquid phases, and chaotic mixing characteristics that can make scale up difficult (Deckwer & Field, 1992). These disadvantages may deter the use of bubble columns for biogas upgrading in cases where more expensive alkaline solvents (e.g. NaOH , CaO) that require regeneration are used, however, since the alkaline effluent used in this study is a byproduct (and, therefore, relatively free), it is believed that the advantages may far outweigh the disadvantages. Three major factors were tested to determine if the selective removal of H_2S over CO_2 from biogas could be enhanced in a simple bubble column reactor: sparger configuration, effluent height, and biogas superficial velocity (flow rate).

The sparger configuration played an important role in selectively removing H_2S because large bubbles decreased the absorption of CO_2 into the effluent. Both H_2S and CO_2 contribute hydronium upon absorption, decreasing the effluent pH over time (see Figure 3.3). For H_2S to be completely removed from biogas a pH above 9 must be maintained. Therefore, resisting the absorption of CO_2 is crucial for selectivity to occur in a bubble column. Significant differences in the volume of biogas that could be purified per volume of effluent can be seen in Figure 3.3 between the use of an airstone sparger (small bubbles) and an inlet orifice sparger (large bubbles). The inlet orifice sparger was able to resist the absorption of CO_2 , which in turn allowed a greater quantity of H_2S to be absorbed. In addition to the two sparger configurations, plastic pall rings were used inside the bubble column to mimic a packed bubble column. This configuration should break up the bubbles and allow for more consistent absorption into the effluent. To the surprise of the researchers, this reduced the H_2S removal efficiency, supporting the conclusion that larger bubbles either promote the absorption of H_2S or decrease the absorption of CO_2 . As Figure 3.3 indicates, the gas-to-liquid ratio, at around 100% removal, improved when the inlet orifice sparger was utilized compared to the airstone sparger and the packed bubble column, providing further evidence that selection was enhanced, by inducing large bubbles in the bubble column.

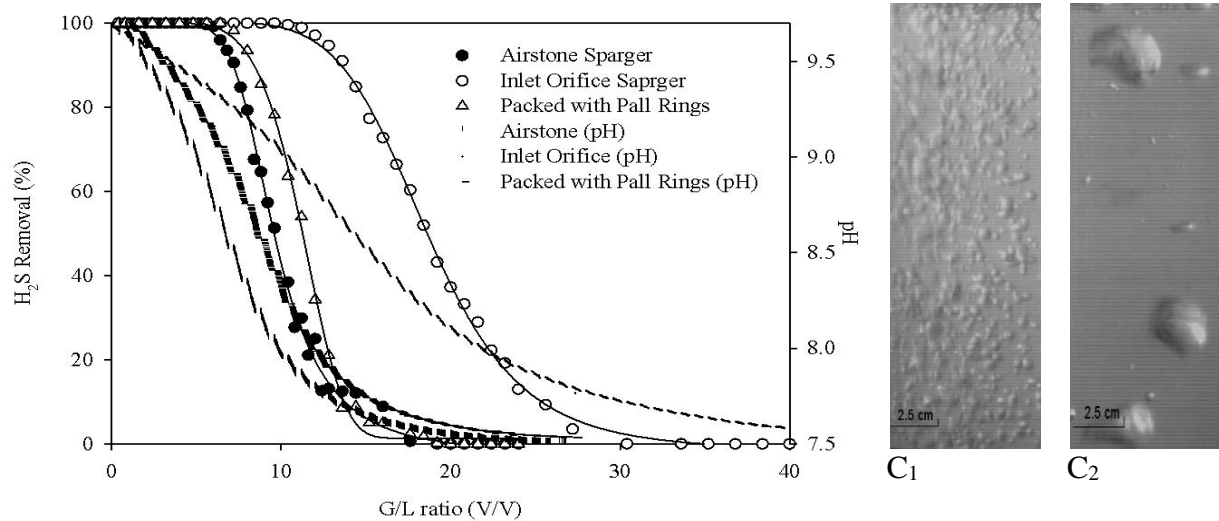


Figure 3.3: H_2S removal efficiency using an airstone sparger (C_1) and inlet orifice (C_2); photographs were taken with a high-speed camera in water

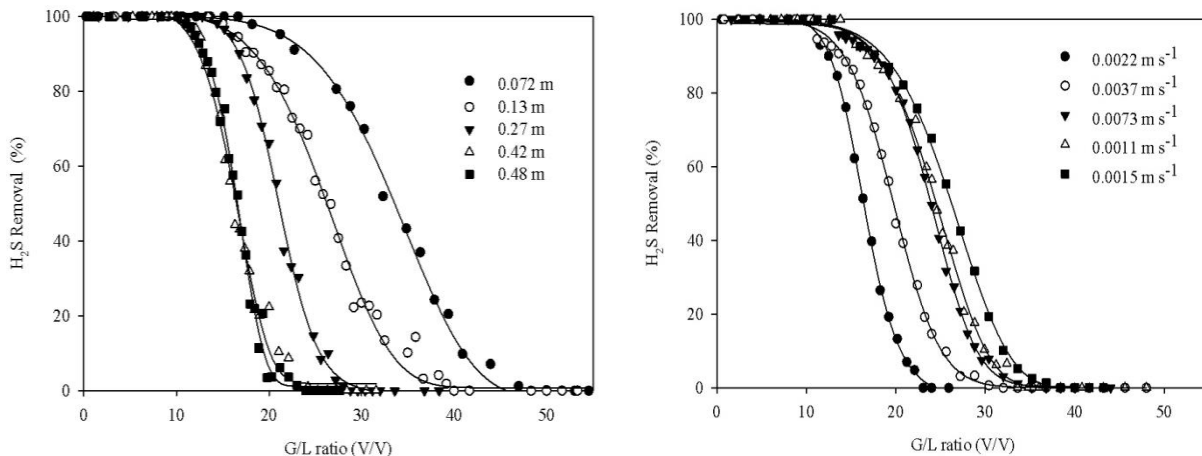


Figure 3.4: Effect of effluent height (*left*) and superficially velocity (*right*) on the H_2S removal efficiency and G/L ratio

Based on these initial results, the inlet orifice sparger (C_2) was used to complete two additional selectivity tests. First, the effluent height was varied while holding the superficial gas velocity constant. Figure 3.4 (left) illustrates that selectivity increased as effluent height decreased. This could be attributed to an increased reaction time between the bubble and the pretreated effluent as the effluent height was increased. This allowed more CO_2 to absorb and thus led to a faster drop in pH.

Second, effluent height was held constant and the superficial velocity was altered. The results indicated that an increase in superficial velocity improved the selective removal of H_2S over CO_2 from biogas (Figure 3.4, right). This result has also been found in other research (Wallin &

Olausson, 1993). The results from these three experiments provide ways to easily increase the volume of biogas being purified of H_2S .

3.3 Conclusions

The experiments conducted by WSU researchers provide compelling support for the use of a high pH liquid for the removal of impurities (e.g. H_2S) present in biogas and, since this media is a byproduct of the NR technology developed at WSU, it is in abundance and relatively free of cost. In AD systems using this commercially proven NH_3 stripping technology, a high pH effluent will be in continual production and due to its alkaline characteristics (pH greater than 9) it can be used to completely strip raw biogas of H_2S . Instead of sending post NR effluent directly to the lagoon for storage, the effluent could be used to upgrade biogas on dairy farms where other technologies (e.g. amine scrubbers, water scrubbers, etc.) are not viable. If RNG is not the main target for biogas, this technique is still a very compelling and practical solution for farmers utilizing gen-sets due to the removal of H_2S before igniting biogas. This could eliminate corrosion and maintenance issues that are currently a significant operating cost at dairy digesters.

In addition, the experimental results showed that expensive and complex absorption apparatuses were not necessary due to the continual production of this high pH effluent from the NR process. Instead a low tech, low cost bubble column could be used and through manipulation of the bubble size (bigger the bubble the better) the selective removal of H_2S over CO_2 can be achieved, thus allowing for more H_2S to be removed from raw biogas per volume liquid and thus increasing the efficiency of the process. One patent has been filed and further scale-up of this technique is currently under investigation for both CO_2 and H_2S removal from raw biogas at a dairy digester located in Chilton, WI.

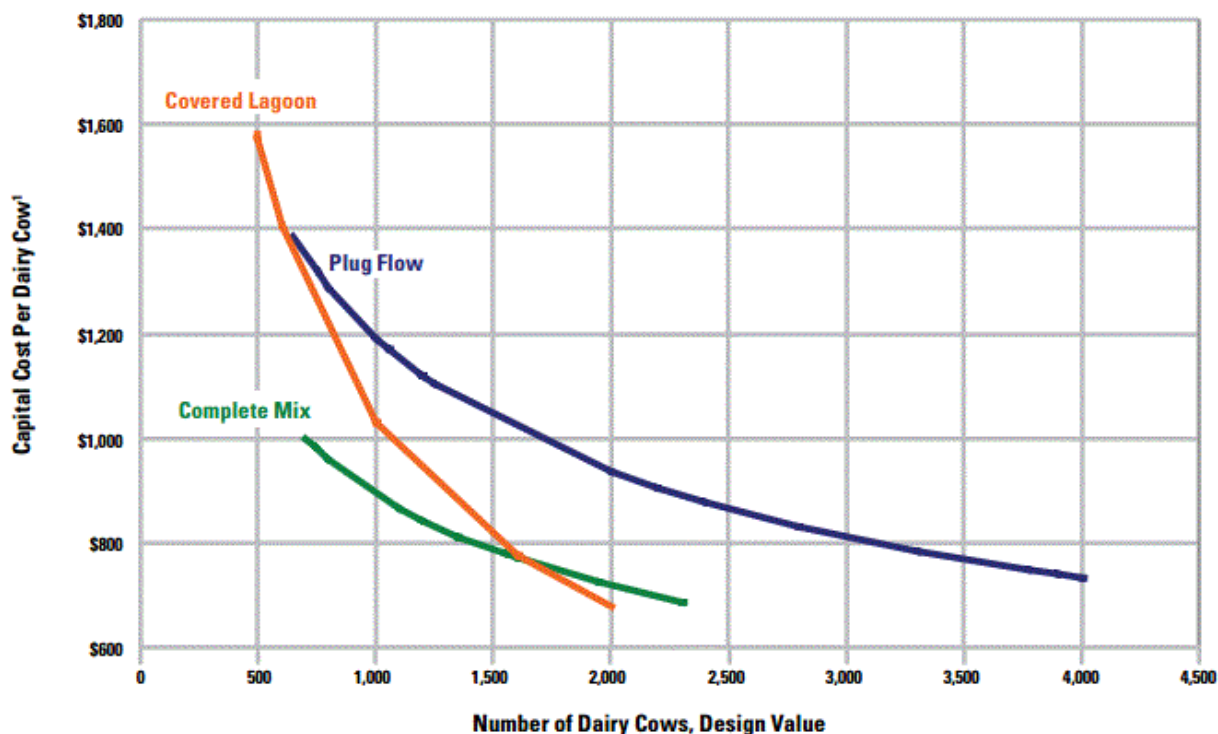
Anaerobic Digestion on Small-sized Dairies

Craig Frear and Chad Kruger

4.1 Introduction

In the U.S., most digesters treating agricultural wastes are located on large-scale dairy operations. EPA AgSTAR has identified the typical characteristics of dairy farms where biogas recovery systems may be profitable to be those dairies (1) with more than 500 animals and (2) with flushed or scraped freestall barns and open lots (AgStar, 2010b).

While some studies suggest that AD is economically viable only on dairies with more than 500 animals, other studies have shown that AD can be viable on livestock operations between 200 and 400 animals (Mehta, 2002). Figure 4.1 shows how the economics of AD hinder the adoption of digesters on small-scale operations.



¹ Costs are stated in September 2009 dollars.

Figure 4.1: Capital cost per dairy cow for complete mix, plug flow, and covered lagoon AD systems (AgStar, 2010a)

As of June 2013, WA had eight operating dairy digesters, all using manure from at least 800 cows. Figure 4.2 shows the location of these digesters and of the state's 417 commercial cow dairies. Small- to medium-sized dairies, those with less than 700 mature dairy animals, make up 75 percent

of these operations. Thus, smaller operations represent a significant potential source of renewable energy that could be generated from agricultural waste. Adoption of AD on these smaller dairies would also contribute to odor reduction, pathogen destruction, chemical oxygen demand (COD) reduction, nutrient removal, and GHG reduction (Frear, 2009).

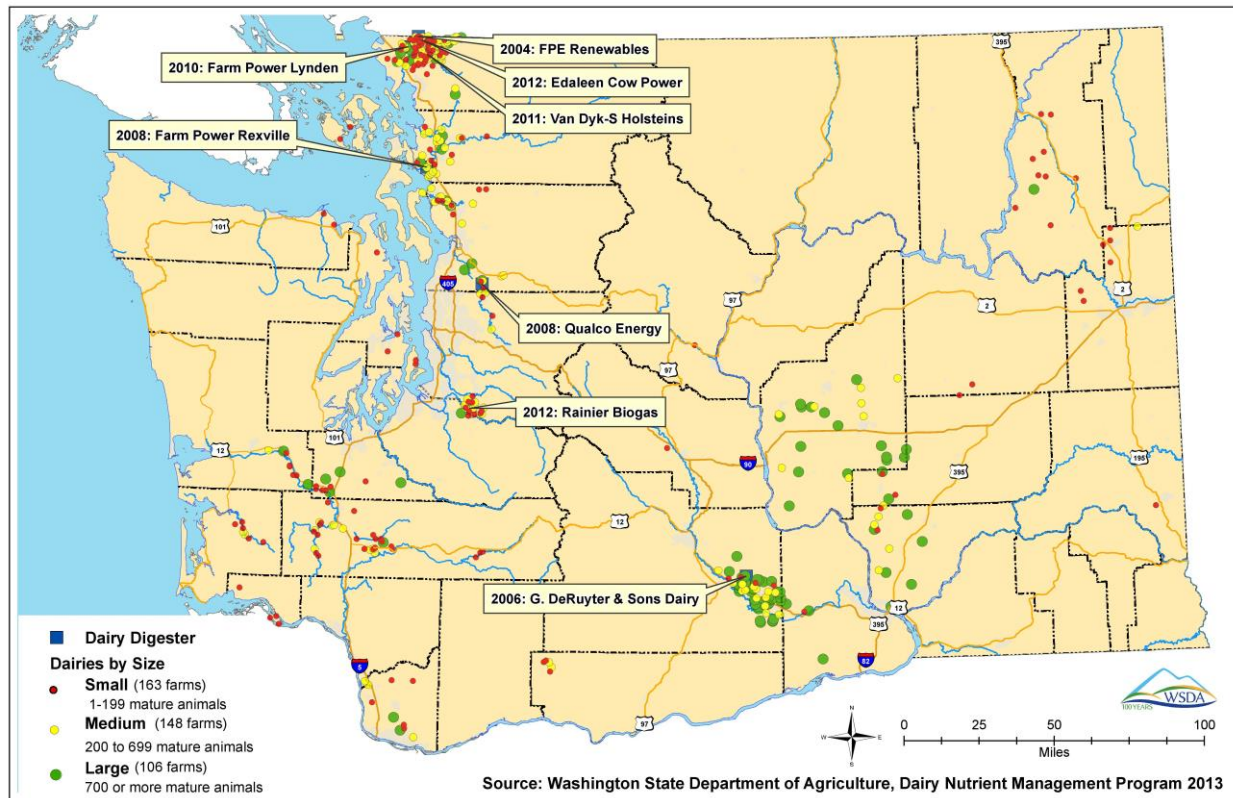


Figure 4.2: Dairy digesters and dairy farms in WA, 2013

These smaller operations will likely not find AD viable if they are counting on revenue from electrical generation or transportation fuels to cover costs. One way to improve the economics of AD for these dairies is to change the end use of the biogas. Converting biogas to electricity requires an internal combustion engine, which is costly (accounting for 50% of the capital costs and 80% of the operating costs of AD operations). Thus, reducing or eliminating this cost could reduce the overall costs considerably. If a dairy digester generated heat rather than electricity, it could use boilers instead of an engine, reducing both capital and operating costs considerably.

Washington has experienced a resurgence of interest in small-scale dairy production and processing. This growth has been encouraged by the increased popularity of artisan cheeses, a push to improve farm economic viability through valued-added products and direct marketing, and a movement that promotes consumption of locally produced raw milk from pasture-fed dairy animals. Between 2002 and 2006, the number of small-scale milk processors in Washington more than doubled to 47 (WSDA, 2006).

In the last seven years, according to WSDA, the number of small-scale milk processors doubled again to 96. Of these milk processors, 85 are also milk producers (licensed dairy farms), split almost evenly between cow and goat milk dairies. These producer/processor dairies need to heat their facilities and also need thermal energy in their milk processing. This need is currently met with natural gas or propane. Using AD to produce heat may represent an attractive option, given the combined benefits in terms of sustainability, marketability, and reductions in existing heating costs as well as the opportunity to control odor and destroy pathogens.

4.2 Demonstration Site and Results

Even given the benefits described above, design improvements to small-scale digesters are needed to further improve project economics for small-scale dairies, as a specific case study shows. Cherry Valley Dairy (CVD), near Seattle, WA, is a small dairy transitioning from a traditional commodity-based model to a sustainable, urban dairy producing specialty products. Like many small dairies, CVD expanded production in the 1990s and early 2000s in an effort to maintain financial viability, which created water quality concerns due to manure management. CVD's new owner is pursuing a new business model for community-based marketing of value-added dairy products, which has resulted in the following changes:

- Significant reduction in the total number of milking animals,
- Construction of an on-farm micro-processing facility for artisanal cheese and butter,
- Plans for on-site sales to the neighboring community, and
- Sales to a local farmers' market and a local cooperative grocery chain.

An important part of the brand identity and value for these sustainable, artisanal dairy products will be the improved environmental footprint of the dairy. With this in mind, CVD has made site design and operational investments to reduce storm water runoff. It is also exploring options for improved manure management and on-site CH₄ capture with an anaerobic digester. In response to this interest, WSU and Andgar Corp. have designed a new AD reactor that could reduce the total "footprint" and cost of AD for small dairies. As described above, the reactor provides thermal energy for heating/chilling in CVD's new micro-processing facility. The system is also designed to accept milk processing wastewater (enhancing biogas productivity). Lastly, it is anticipated that the soil amendment products recovered from the digester can be marketed to local gardeners on-site or through the farmers' market and co-op as high-quality "co-products."

This model represents a potentially sustainable system that could reduce CVD's fossil energy use and CH₄ emissions and improve water quality to complete the "sustainability story" necessary to add value to CVD's model for sustainably produced dairy products. The objective of this task was to install a pilot AD unit at CVD, proceed with start-up and training of the CVD staff to manage the digester, and operate it for one calendar year as a basis for future evaluation of the unit.

The unit was installed and began operation in early July of 2012 (Figure 4.3). By mid-July, it was operating at a temperature of around 38°C and producing biogas. The digester received 200 gallons per day of manure from the manure collection pit, and periodic doses of wash water from the milk processing operation. Biogas was captured and flared. The gas volume and makeup was monitored to evaluate whether it was feasible to use the biogas as a renewable energy source at the dairy.



Figure 4.3: Small-scale vertical mixed plug flow digester with a hot water heater for heat generation located CVD near Seattle, WA

4.3 Conclusions

As of March 2013, the unit was generating approximately 100 cubic feet of biogas per day. Unfortunately, the biogas quality was rather poor due to a low concentration of CH_4 (10%). Possible reasons for poor quality gas production and CH_4 content include inconsistent feeding of manure/wash water and problems maintaining temperature through the winter. The team is currently seeking additional funding for further evaluation of management, financial, and environmental implications.

Pretreatment of Fibrous Feedstock for Entry into Digester

Mahesh Bule, Allan Gao, Liang Yu and Shulin Chen

5.1 Introduction

In the PNW, AD is primarily used to treat municipal and animal wastewater, although it is also potentially applicable to lawn clippings, field residues (e.g. wheat and barley straw or hay), or dedicated crops (e.g. corn or wheat). Table 5.1 shows the CH₄ potential of various plants and plant materials that have been investigated. A relatively recent inventory indicated that there was more than 2 million dry tons of lignocellulosic residue available for conversion to energy through AD or other processes in WA (Frear et al., 2005).

Table 5.1: CH₄ yield from the AD of different plant material (Braun et al., 2010)

CH ₄ Yield (m ³ per t VS)			
Maize (whole crop)	205–450	Barley	353–658
Wheat (grain)	384–426	Triticale	337–555
Oats (grain)	250–295	Sorghum	295–372
Rye (grain)	283–492	Alfalfa	340–500
Grass	298–467	Sudan grass	213–303
Clover grass	290–390	Reed canary grass	340–430
Red clover	300–350	Ryegrass	390–410
Hemp	355–409	Nettle	120–420
Flax	212	Miscanthus	179–218
Sunflower	154–400	Rhubarb	320–490
Oilseed rape	240–340	Turnip	314
Jerusalem artichoke	300–370	Kale	240–334
Peas	390	Chaff	270–316
Potatoes	276–400	Straw	242–324
Sugar beet	236–381	Leaves	417–453
Fodder beet	420–500		

In Europe, 90% of farm-based digesters operate using energy crops due to higher biomass potential compared to waste-based digesters (DeBruyn et al., 2006). In Germany, the number of systems digesting energy crops rose from 100 in 1990 to nearly 4,000 in 2008 (Braun et al., 2010). Most of the large-scale digesters utilizing dedicated crops (either with or without other substrates) have no pretreatment step prior to AD. Instead, they use an extended HRT to break down recalcitrant wastes and achieve CH₄ potentials. In Austria, 41 full-scale energy crop digestion plants had a median HRT of 133 days (Braun et al., 2010). High HRT raises capital costs, but these projects

are still feasible in Europe due to subsidies and high received electrical sale prices (DeBruyn et al., 2006). In the U.S. context, where received electrical prices are much lower, alternative methods, such as pretreatment, must be explored before lignocellulosic residues will be utilized in AD.

Even in the existing digesters that use dairy waste, nearly half of the organic matter is comprised of lignocellulosic material (e.g. dairy fiber) representing a large potential source of carbohydrates that could be converted into usable intermediates of mono-sugars (Jungersen & Ahring, 1994; Yue et al., 2011). These recalcitrant lignocellulosic materials are difficult to digest, lowering the biogas potential of such projects. This is one reason why more readily degradable organics have often been co-digested with manure to enhance to biogas production (WSDA, 2011).

5.1.1 Pretreatment of Lignocellulosic Material

Pretreatment of recalcitrant materials can improve the efficiency of the AD process. This approach becomes particularly important when utilizing biomass that contains a high concentration of lignocellulose. The lignin provides a protective barrier around the hemicellulose and cellulose contained in the plant cell wall, preventing easy hydrolysis. Pretreatment techniques can fractionate the physical and chemical structure, resulting in more efficient hydrolysis and increasing downstream CH₄ production (Liao et al., 2008). Table 5.1 illustrates the structure of lignocellulosic material and shows how lignin provides a protective barrier that prevents plant cell destruction by bacteria; also included is a schematic of how pretreatment can aid in unlocking the hemicellulose and cellulose fractions (Kumar et al., 2009).

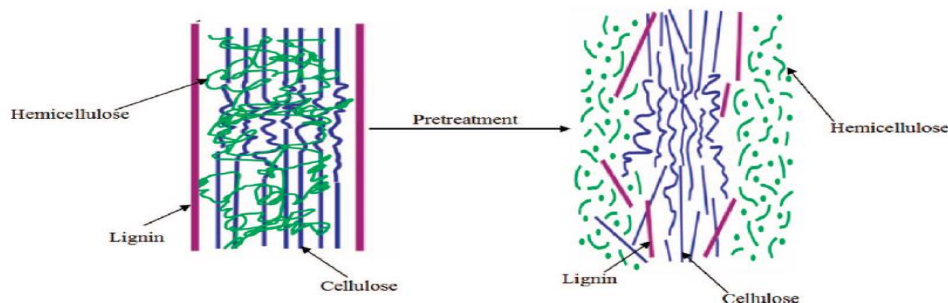


Figure 5.1: Schematic of the role of pretreatment in the conversion of biomass to fuel (Kumar et al., 2009)

Numerous pretreatment techniques have been extensively studied for their application to cellulosic ethanol production: ammonia fiber expansion (AFEX), steam explosion, hydrothermal, and dilute acid treatment. These pretreatments are able to separate lignin from the lignocellulosic matrix (Gao, 2012). Table 5.2 shows the commonly used process parameters used for different pretreatment techniques. As the high temperatures and pressures associated with each of these techniques indicate, these pretreatment techniques require high energy inputs and also require high capital costs (Hill et al., 2006; Himmel et al., 2007). If acids or bases are used, as in many of the techniques mentioned in Table 5.2, then reactors need to be highly corrosion-resistant. This generally requires nickel alloy materials, further increasing the capital costs of pretreatment.

Table 5.2: Commonly used parameters for different pretreatment techniques reported in the literature (Gao, 2012)

	Chemical Usage/ton biomass	Temp (°C)	Pressure (bar)	Residenc e Time (min)	Glucan Yield (%)	Xylan Yield (%)
Organosolv	4:1 C ₂ H ₅ OH	90–220	14–20	25–100	85–100	n/a
Ionic Liquid	10:1 Ionic liquid	100–150	n/a	30–120	55–97	n/a
Dilute Acid	0.03:1 H ₂ SO ₄	160–220	5–15	2–30	85–95	70–95
Steam Explosion	0.005:1 H ₂ SO ₄	160–290	20–24	5–15	85–100	85–95
Liquid Hot Water	6:1 H ₂ O	160–230	24–27	15–20	55–90	80–95
AFEX	1:1 NH ₃	60–140	20–41	5–15	55–100	55–95
Alkaline	1.2 MPa O ₂	170–220	5–12	15	70–80	50–55
Lime	0.75:1 Ca(OH) ₂	25–150	2–20	1 week	>90	80

A less-severe pretreatment process which avoids harsh chemicals would lower the capital costs of pretreatment (Gao, 2012). Low pressure/temperature pretreatments that do not require corrosion-resistant vessels have been studied for degradation of the lignin fraction in lignocellulosic material (García-Cubero et al., 2009; Kim et al., 2009). Two techniques, ozone and soaking aqueous ammonia (SAA), have been shown to be effective pretreatments for degrading or removing lignin (Gao, 2012; Gao et al., 2012). SAA is able to retain the hemicellulose fraction while removing the lignin fraction (Kim & Lee, 2006). This attribute is important to AD because studies have shown that anaerobic bacteria are quite capable of utilizing hemicellulose in addition to cellulose (Liew, 2011). Also, little to no modification occurs to lignin but SAA is able to separate lignin from the matrix and allow greater access to the cellulose and hemicellulose fraction, ultimately allowing for more efficient enzymatic hydrolysis (Gao, 2012). The use of ammonia on lignocellulosic material has also been shown to cause a structural shift from cellulose I molecules to cellulose III molecules, which allows greater accessibility for anaerobic cellulose degradation due to greater internal surface area (Gao, 2012).

Ozone pretreatment has been shown to be effective due to ozone's strong oxidizing action, which allows it to attack compounds incorporating conjugated double bonds and functional groups with high electron bonds. It is very effective at targeting lignin although some hemicellulose is degraded as well (García-Cubero et al., 2009). Other advantages include the fact that ozone does not produce inhibitory degradation products that could interfere with hydrolysis, and the fact that reactions occur at ambient temperature and pressure (García-Cubero et al., 2009).

However, disadvantages do exist with both of these pretreatment techniques. SAA pretreatment requires a long time (up to 24 hrs) to achieve acceptable lignin degradation/separation. Ozone pretreatment can take up to 2 hrs to complete, which is an improvement over SAA. However, it is

a more costly pretreatment process than SAA because of the high energy requirements for producing ozone (Gao, 2012). In an attempt to overcome these key disadvantages, the two treatments were combined as well as tested separately (Gao, 2012; Gao et al., 2012).

5.1.2 Pretreatment and Anaerobic Digestion

Cellulose has a highly uniform composition consisting of up to 10,000 D-glucose molecules, which are connected by β -1,4 bonds and tilted 180 degrees towards their neighbors, producing a uniform crystalline structure of microfibrils (Schwarz, 2001). This crystalline structure combined with bonding to other polymers, such as hemicellulose and lignin, creates an unusually resilient substrate for hydrolytic enzymes (Schwarz, 2001). Anaerobic organisms are able to handle cellulose differently than aerobic microorganisms. Aerobic microorganisms like fungi produce single enzyme components, which are connected to binding modules (Schwarz, 2001). On the other hand, anaerobic cellulose-degrading bacteria have a unique enzyme complex called a cellulosome that is responsible for their cellulolytic behavior. The cellulosome differs from free cellulase units because the cellulosome is composed of a primary scaffolding subunit that allows for the combination of up to 11 enzymes, creating a very efficient process for degrading cellulose and hemicellulose (Bayer et al., 2007). On the cell surface, these multi-protein and multi-functional enzymes appear as polycellulosomal aggregates, which promotes the adherence of the bacterium to cellulose (Leschine, 1995). Figure 5.2 provides a hypothetical schematic of what a cellulosome looks like as it attaches to cellulose.

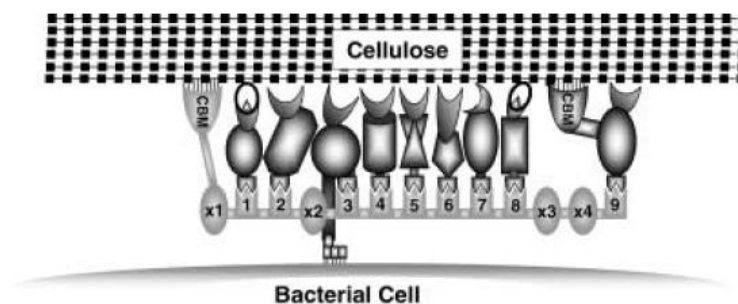


Figure 5.2: The structure of a cellulosome (Schwarz, 2001)

This close proximity between the cell and the substrate minimizes the diffusion losses of the hydrolytic products, which is thought to be a major advantage for attached cells. Due to the multi-enzyme approach of cellulosomes, there may exist four distinct advantages for the successful degradation of cellulose, including the following (Schwarz, 2001):

1. Synergism is optimized by the correct ratio between the components, which is determined by the composition of the complex
2. Non-productive adsorption is avoided by the optimal spacing of components working together in synergistic fashion
3. Competitiveness in binding to a limited number of binding sites is avoided by binding the whole complex to a single site through a strong binding domain with low specificity
4. A halt in hydrolysis on depletion of one structural type of cellulose at the site of adsorption is avoided by the presence of other enzymes with different specificity

Thus, cellulosomes have many advantages over single enzymes for degrading cellulose in plant material. On the other hand, some disadvantages are present as well. One disadvantage when compared to fungi enzymes is that cellulosome is not a free enzyme, and is unable to diffuse through media whereas fungal enzymes are aqueous free enzymes, greatly increasing their kinetic rate. In addition, the hydrolysis step can be an extremely long process for the cellulosome due to the complex matrix of the lignocellulosic material. The crystalline structure of cellulose and the envelopment of cellulose and hemicellulose by lignin slow down the degradation of lignocellulose to produce sugars. This results in a longer HRT, going from days to many months, for hydrolysis to proceed and subsequent methanogenic activity to occur (Keating et al., 2013).

To optimize the cellulosome ability to degrade lignocellulosic biomass, extensive research into the hydrolytic mechanism is necessary, with respect to the substrate-binding capacity and specificity, interplay of catalytic components and their distribution and composition, end product inhibition, and stability against proteases or denaturation (Schwarz, 2001). Due to these unresolved mechanisms, commercialization of highly efficient cellulosomes cannot be relied upon as an immediate solution to the AD of lignocellulosic biomass. However, if the cellulose can be removed from the crystalline structure, thus releasing single cellulose units, the cellulosome will have greater access to degrade biomass to soluble organic matter for acidogenic bacteria to convert to hydrogen and acetate, and ultimately for methanogens to convert to CH₄. Therefore, pretreatment techniques can bridge this gap between slow hydrolysis and cellulosome optimization to enhance hydrolysis and biogas production.

Extensive studies have been performed to evaluate the effect of different pretreatments on agricultural wastes and wastewater for increased biogas production. Pretreatment methods include biological and chemical additives (Lettinga et al., 1980), thermochemical pretreatment of water hyacinth (Patel et al., 1993), ultrasonic pretreatment of waste-activated sludge (Wang et al., 1999), acid hydrolysis of dairy manure fiber (Liao et al., 2006), catalytic liquefaction of dairy manure (Jungersen & Ahring, 1994), and dilute acid and dilute sodium hydroxide pretreatment of dairy manure fiber (Yue et al., 2011). The effect that pretreatment techniques have on biogas production varies considerably. One technique, wet explosion of dairy manure fiber, increased the biogas production by 75% (Biswas et al., 2012) while other techniques using different feedstocks have resulted in increases ranging from 28.6–225.6% (Chandra et al., 2012; He et al., 2009). Nevertheless, results have shown that pretreating agriculture waste before digestion is beneficial for biogas production (Chandra et al., 2012; He et al., 2009). However, many of these techniques require high pressure and temperature, resulting in high energy inputs and increased costs. Due to these high energy requirements, the pretreatment techniques have not been widely used on a commercial scale.

Due to high lignin separation/removal and low temperature/pressure requirement, ozone and SAA were investigated as a pretreatment technique for grass clippings. In addition, a combination of the two pretreatments, known as ozone and soaking aqueous ammonia (OSAA), was tested. Initial tests were performed on grass clippings with future experiments on the fiber contained in dairy manure planned.

Work in this area achieved the following objectives:

1. Characterization of lawn grass pre- and post-pretreatment
2. Developing pretreatment technologies for improved enzymatic hydrolysis
3. Evaluating CH₄ production from untreated and pretreated lawn grass

5.2 Methods and Results

5.2.1 Sample Preparation and Compositional Analysis

Lawn grass was obtained locally and hammer milled. For ozone pretreatment, the grass samples were sieved through a 32–42 mesh Tyler Standard Screen Scale. Ozone was produced by a L11-L24 Ozone Generator manufactured by Pacific Ozone, California. 3 g of lawn grass particles were adjusted with moisture (90% w/w) and placed into an enclosed stainless steel reactor. The ozonation reaction was performed under 5.3% ozone concentration (5.3% w/w) at a flow rate of 2 L/min for 10 mins. During the SAA pretreatment process, the lawn grass particles were pretreated by SAA using 28–30% (w/w) ammonium hydroxide solution (JTB-9721-03 Ammonium Hydroxide 28–30%) with a solid-to-liquid ratio of 2:10 at 50°C for 24 hrs with no agitation in 1,000 ml screw cap Pyrex solution bottle. The sequential ozone and SAA process used the same procedures of each individual pretreatment described above but at different time intervals. Grass samples underwent ozonation for 10 mins followed by SAA for 6 hrs.

Afterwards, compositional analyses were performed on untreated and pretreated lawn grass for determination of structural carbohydrates in biomass. Results from the compositional analysis (Figure 5.3) showed decreases in sugar concentration after ozone pretreatment while degrading minimal amounts of cellulose. The SAA and OSAA pretreatment increased sugar content, which could be due to removal of ash, extractives and conversion of insoluble lignin to soluble lignin. Any increase in sugar content will lead to a better hydrolysis process and could improve biomethane production. Based on these compositional analyses, the sequential OSAA pretreatment was the best technique for degrading/removing lignin and should allow anaerobic bacteria greater access to cellulose and hemicellulose during the hydrolysis step in AD.

Table 5.3: Compositional analysis of untreated and pretreated grass

Components	Untreated (% w/w)	Ozone (% w/w)	SAA (% w/w)	OSAA (% w/w)
Sugars				
Arabinose	2.90 ± 0.08	1.90 ± 0.01	3.70 ± 0.01	4.35 ± 0.36
Galactose	1.23 ± 0.02	0.89 ± 0.01	1.28 ± 0.01	0.83 ± 0.02
Glucose	16.91 ± 1.07	16.14 ± 0.11	30.85 ± 0.02	30.96 ± 0.13
Xylose	9.52 ± 0.50	9.65 ± 0.06	10.73 ± 0.04	10.59 ± 0.04
Total	30.55 ± 0.46	28.58 ± 0.20	46.55 ± 0.05	46.73 ± 0.54
Lignin				
Acid insoluble	16.66 ± 0.57	14.21 ± 0.13	13.41 ± 0.86	11.13 ± 1.01
Acid soluble	3.73 ± 0.09	4.81 ± 0.72	1.81 ± 0.34	2.51 ± 0.47
Extractives				
Ethanol	5.83 ± 0.21	ND	ND	ND
Water	4.18 ± 0.39	ND	ND	ND
Total proteins	1.52 ± 0.002	ND	ND	ND

ND: Not determined

5.2.2 Developing Pretreatment Technologies for Improved Enzymatic Hydrolysis

To confirm these initial findings, enzymatic hydrolysis was used to investigate sugar recovery from untreated and pretreated grass samples. The enzymatic hydrolysis of pretreated lawn grass was performed at 1% (w/v) solid loading in 0.05 M sodium citrate buffer (pH 4.8) containing 100 µl 2% sodium azide with 30 FPU/g of cellulase (Novozymes NS 50013) and 30 CBU/g of β-glycosidase (Novozymes NS 50010). Table 5.4 shows the sugary recovery from untreated grass, along with the three pretreatments. Results indicated that cellulolytic enzymes were unable to hydrolyze sugars from untreated grass samples, which is likely to result in low biogas production during methanogenesis. Ozone pretreatment improved sugar recovery to 48.50%, while SAA further improved sugar recovery to 86.71%. However, the highest sugar recovery (89.63%) was achieved with the combination of 10 mins of ozone pretreatment and 6 hrs of SAA pretreatment, while minimizing the cost and time required.

Table 5.4: Sugar recovery of lawn grass after different pretreatments

Sample	Sugar recovery (%)
Untreated	0.0 ± 0.00
Ozone Pretreatment	48.50 ± 2.17
SAA Pretreatment	86.71 ± 0.20
OSAA	89.63 ± 2.09

5.2.3 Utilizing Untreated and Pretreated Grass Clippings for Methane Production

To fully understand how biogas production was affected by pretreatment, untreated and pretreated grass was anaerobically digested to determine the biomethane production and change in VFA. Figure 5.3 depicts the biomethane production of the different treatments over 30 days.

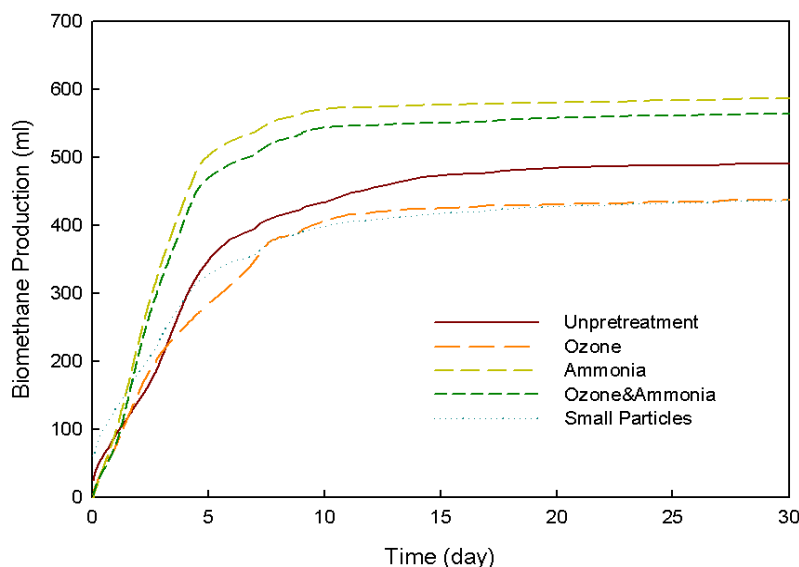


Figure 5.3: Biomethane productions of different grass treatments

The results indicated significantly higher biomethane production from SAA and OSAA pretreatment compared to the ozone pretreatment or untreated samples. Ozone pretreatment negatively impacted biomethane production compared to untreated grass. This could be due to the low sugar yield that occurred from the short ozonation process. In comparison to untreated grass samples, ozone pretreatment actually caused a loss of sugar. One study on canned maize production sludge showed the biomethane production using ozone pretreatment also resulted in a lower biogas production when ozone pretreatment was performed for 30 mins (Beszedes et al., 2009). However, when the process was increased to 60 mins, the biomethane production increased around 28% (Beszedes et al., 2009). Based on these other findings, it is possible that increasing the ozonation process beyond 10 mins would also increase biomethane production, though this would also increase the energy required for ozonation. It is also possible that the ozone pretreatment inhibited methanogenesis.

When the SAA pretreatment was utilized, biomethane production increased nearly 30% compared to the untreated grass samples. OSAA also resulted in satisfactory biomethane increases (around 18%), though there are indications that the OSAA pretreatment inhibited methanogenesis somewhat.

5.3 Conclusions

This study provided concrete evidence that CH_4 production was enhanced when applying pretreatment techniques to lignocellulosic biomass. Overall, the authors suggest SAA may be the best pretreatment because this process could integrate with the NR process being developed at

WSU. Specifically, the ammonia would be available without cost as a byproduct of NR, and can be recycled and used for the production of bio-fertilizers after it is used for pretreatment. Another positive attribute of the SSA process is that it does not require any special handling, making it easier to integrate than the OSAA pretreatment process. However, a further techno-economical analysis will be required to develop this integrated process.

The experiments performed here provide a framework for future work to scale up pretreatments. In addition to scale up efforts, a logical next step in this research would be to test these pretreatments on dairy manure fiber. If these pretreatments could boost biomethane production from the currently underutilized lignocellulosic material within dairy manure, it could boost economics at the existing AD facilities on dairies in the PNW.

Anaerobic Digestion of Algal Biomass Residues with Nutrient Recycle

Baisuo Zhao, Jingwei Ma, Quanbao Zhao, Craig Frear and Shulin Chen

6.1 Introduction

Microalgae are considered one of the most promising feedstocks for biofuel production due to their high growth rates (Scott et al., 2010) and high concentration of lipids compared to other feedstocks (e.g. corn, maize, hemp, soybean, jatropha, camelina, palm oil) (Mata et al., 2010). The lipids contained within the cells of microalgae can be processed into a multitude of energy products including transesterified biodiesel (Chisti, 2007); fermented bioethanol (Bush & Hall, 2006); photo-biological hydrogen (Ghirardi, 2000); hydrocarbon biofuels for drop-in replacements of gasoline, diesel, and jet fuel (Jones & Mayfield, 2012); and CHP or RNG via anaerobically generated CH_4 (Sialve et al., 2009; Uellendahl & Ahring, 2010). Although the bulk of this research is oriented towards biodiesel production, anaerobically digesting microalgae for CH_4 generation has also garnered considerable interest.

The more simple and lower-cost AD pathway may be particularly appropriate for processing the lower-lipid algae that are cultivated in blooms in open ponds or waterways, often for wastewater treatment and environmental protection purposes (Sialve et al., 2009). In addition, a large quantity of algae residue will be generated at algal bio-refineries composed of $\geq 50\%$ lipid content by dry weight (Scott et al., 2010), which will require disposal. Both of these materials can be viewed as attractive feedstocks for AD (Davis et al., 2011; Delrue et al., 2012; Sialve et al., 2009).

With this in mind, researchers at WSU, working with the National Renewable Energy Laboratory (NREL), investigated how anaerobic microorganisms can digest microalgae either as a whole cell or as an extracted residue for the production of biogas. Figure 6.1 illustrates the integration of algae cultivation and AD utilizing the whole cell or algal residue. Once AD has been completed, the nutrient-rich effluent can be recycled back to the raceway ponds since AD converts N and P from their organic forms to more readily useable inorganic forms.

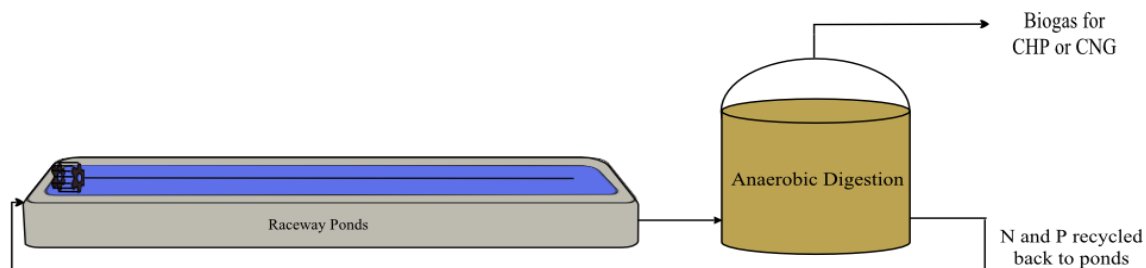


Figure 6.1: Algae cultivation with AD and nutrient recycle—either whole cell or residue

6.2 Methods and Results

6.2.1 Whole Cell Microalgae and Lipid-extracted Microalgae Residue

WSU was one partner of a multi-institutional consortium headed by the Department of Energy. WSU's two key objectives in this consortium included (1) extensive biochemical methane potential (BMP) studies on delivered whole cell and extracted algal residue, with various degrees of biomass pretreatment and (2) translation of the BMP data and capabilities into continuous digestion operation for determination of system capabilities upon scale-up to a 5 L reactor size.

Five model strains, similar to those generated in pilot and commercial algal fuel production facilities, were selected for their diversity, tolerable growth conditions, lipid and protein contents, and other distinct physiological features. Each strain was studied in both whole cell and extracted residue form, generating ten specific algal biomass pretreatments. The five strains that were chosen for this project included *Chlorella vulgaris* UTEX 395 (C), *Phaeodactylum tricornutum* CCMP 632 (P) (provided by NREL), *Nannochloropsis* sp. (N), *Nannochloropsis salina* (NS), and *Nanofrustulum* sp. (NF) (provided by Seambiotic, Solix Biosystems, and Cellana, respectively)—with the five representative of three general algal classes—*Eustigmatophyceae* (N and NS), *Bacillariophyceae* (P and NF), and *Chlorophyceae* (C). Outdoor production systems were used to grow N1 (raceway), NS1 (photo-bioreactor), and NF1 (raceway), with limited information on growth conditions available due to proprietary relations. All microalgae were harvested and centrifuged at room temperature. Subsequently, the biomass were dried and frozen at -20°C prior to lipid extraction or for AD treatment. Five different solvent systems including (chloroform/methanol (2:1 v/v) (control), hexane/isopropanol (3:2 v/v), dichloroethane/methanol (1:1 v/v), dichloroethane/ethanol (1:1 v/v), and acetone/dichloromethane (1:1 v/v)), were evaluated for possible BMP inhibition using whole cell N1 biomass.

6.2.2 Bench-scale and Scale-up Microalgae Digestion

The specific methane productivity (SMP; $\text{L CH}_4 \text{ g VS}^{-1} \text{ fed}^{-1}$) and other AD performance parameters for the five industrial strains evaluated were within a relatively tight range. Table 6.1 summarizes the data from BMP evaluation of the ten different microalgae/treatments. This contrasts with a review of the literature, which indicated a range of CH_4 production of $0.09\text{--}0.54 \text{ L CH}_4 \text{ g}^{-1}$ (Nallathambi Gunaseelan, 1997; Park & Li, 2012; Sialve et al., 2009; Uellendahl & Ahring, 2010).

It is believed that control of a viable inoculum to substrate (I/S) ratio (1.0 VS/VS) during BMP and continuous digestion was instrumental in overcoming long chain fatty acid (LCFA) inhibition and providing for a tighter and more effective range of SMP ($0.304\text{--}0.557 \text{ L CH}_4 \text{ g VS}^{-1}$) and VS reduction (59.33–78.50%). SMP appeared to be less related to algal species and more related to LCFA content within the biomass, with a linear relationship between SMP and ash-free lipid content being developed for easier, less time-consuming determination of approximate SMP for particular biomass strains grown. Figure 6.2 shows this linear relationship between SMP and ash-free lipid content. While not a perfect relationship ($R^2 = 0.814$), it is clear that whole cell or residual biomass lipid content is one of the most important parameters dictating SMP. Presumably, as algal refineries come closer to reality, lipid-extraction processes will become more effective, leading estimated SMP to be a bit lower within the developed range. More importantly, as BMP studies on microalgae biomass can be costly and time-consuming, narrowing of the SMP range with a

suitable linear equation that describes the relationship to remaining lipid content can enhance efficiency.

Table 6.1: BMP and performance indicators for studied biomass

Algae biomass	C1	C2	N1	N2	NF1	NF2	NS1	NS2	P1	P2
Biogas (L Biogas)	5.28	5.31	5.36	5.90	6.93	4.37	8.36	5.11	5.20	4.89
CH ₄ Prod. (L CH ₄)	3.37	3.14	3.57	3.99	5.07	3.04	5.57	3.83	3.37	3.39
CH ₄ Fraction (%)	63.82	59.13	66.60	67.62	73.16	69.56	66.63	74.95	64.81	69.32
95% CH ₄ Prod. (D) ¹	9.75	12.04	5.58	13.94	11.36	9.89	12.71	7.84	13.49	9.70
Max CH ₄ (LCH ₄ /L D) ²	0.046	0.037	0.087	0.037	0.072	0.056	0.074	0.054	0.040	0.050
1° Hydrolysis (K _{Hz}) ³	0.22	0.19	0.41	0.17	0.22	0.31	0.16	0.24	0.16	0.23
Effluent pH	7.17	7.50	7.16	7.05	7.16	7.10	6.94	6.94	7.28	7.45
TAN (mg N/L)	401	865	458	658	322	509	228	326	613	690
Total VFA (mg/L)	250	177	ND	ND	ND	118	151	155	190	55
VS Reduction (%)	66.06	64.21	65.90	64.41	76.41	59.33	78.50	73.83	70.60	60.20
SMP (L CH ₄ /g VS fed)	0.337	0.314	0.357	0.399	0.507	0.304	0.557	0.383	0.337	0.339
TMPE (L CH ₄ /g VS d)	0.510	0.489	0.542	0.619	0.663	0.512	0.710	0.519	0.477	0.563
TMPT (L CH ₄ /g VS) ⁴	0.604	0.552	0.682	0.531	0.882	0.457	0.749	0.598	0.629	0.580
Degradation (%)	55.76	60.19	52.36	75.09	57.47	66.49	74.33	64.06	53.54	58.48

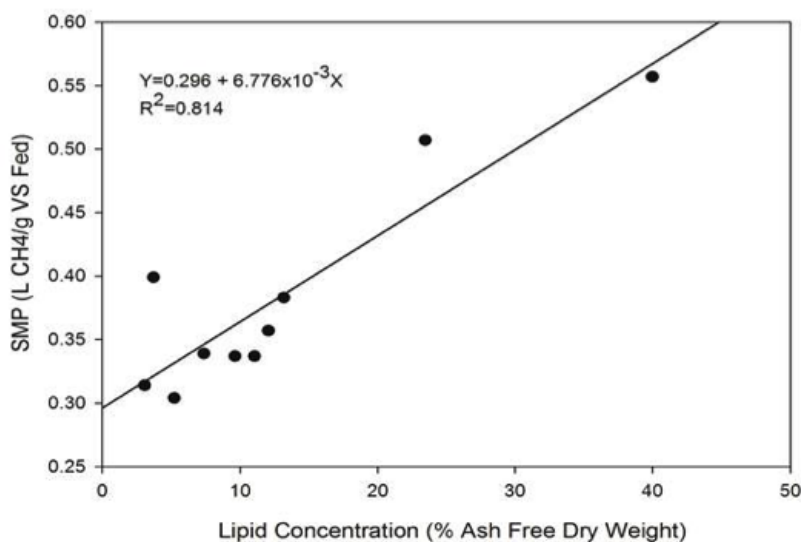


Figure 6.2: Relationship between ash-free lipid content and SMP for microalgae biomass

Several subsequent experiments illustrated the vulnerability of microalgae AD to LCFA inhibition if appropriate process controls are not in place. Figure 6.3 illustrates the effect the I/S ratio had on biogas production for NS1 and NS2. The threshold I/S ratio of 1.0 is consistent with literature.

Hashimoto et al. (1989) determined that a minimum ratio of 0.5 was required for straw digestion at concentrations of 10–40 g VS L⁻¹. Furthermore, Hashimoto showed that maximum CH₄ production rates were achieved when I/S ratios reached 2.0. Owen et al. (1979) and Chynoweth et al. (1993) showed similar results and suggested I/S ratios of 1.0 and 2.0, respectively. As noted, for NS1 a minimum I/S ratio of 1.0 was required to achieve stable digestion, while for NS2, the required I/S ratio was only 0.5. Notably, NS2 has considerably less lipids and therefore less LCFA due to the extraction process. It was thus not exposing the AD microbes to as high a concentration of surface-attaching chemicals. Conversely, the higher concentrations of LCFA in NS1 presumably induced a greater degree of surface adhesion and therefore microbial inhibition, requiring a greater inoculum concentration to overcome this phenomenon.

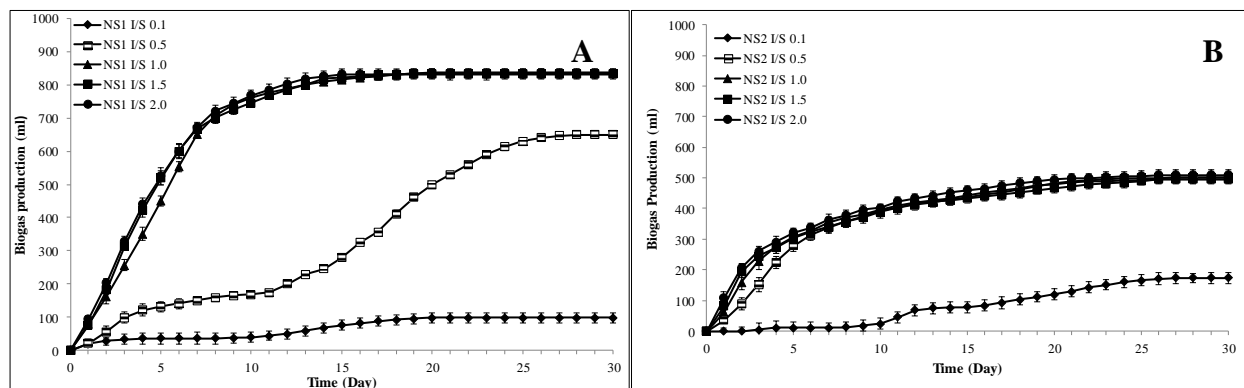


Figure 6.3: Biogas production curves for digestion of NS1 (A) and NS2 (B) at various I/S ratios

Figure 6.4 illustrates how Ca affected the biogas production during digestion of NS1 and NS2. Dosing the system with Ca raised biogas production. We hypothesize that the introduction of suitable concentrations of Ca allowed for a Ca-LCFA substrate that relieved the bacterial biomass of the aforementioned cell-surface inhibition. The effect of Ca dosage concentration appears to solidify this explanation as the lower 0.5X dosage still showed some degree of inhibition before 200 hrs, while 1.0X and 2.0X dosage showed no signs. Thus, Ca may be a useful treatment that can reduce inhibition if needed.

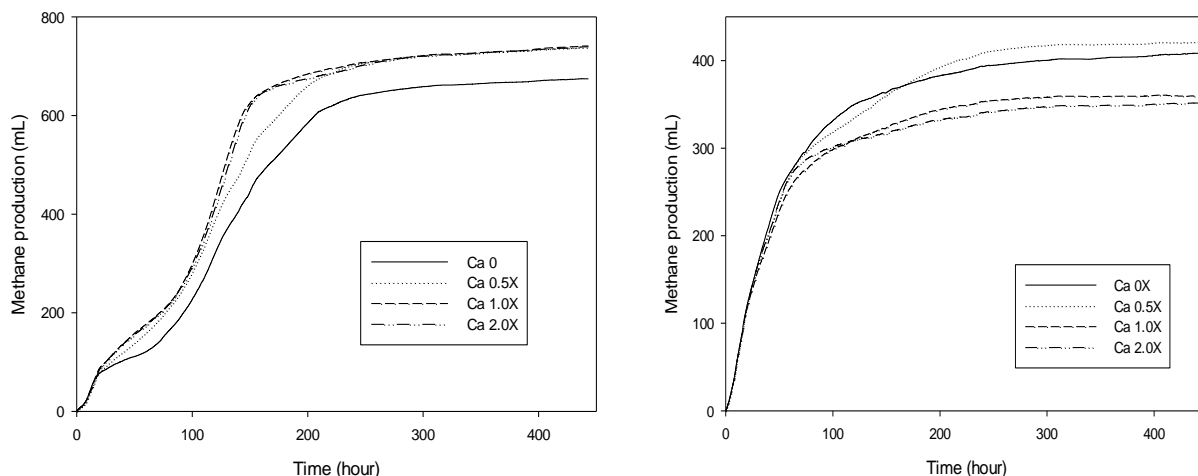


Figure 6.4: Effects of dosing Ca on CH₄ production from NS1 (*left*) and NS2 biomass (*right*)

In the case of continuous digestion, a high I/S ratio was achieved through use of SBR processing, allowing for a much higher biomass concentration. Previous concerns related to carbon-to-nitrogen (C:N) ratio, ammonia toxicity, reduced CH₄ percentages, and poor access to organic material via cellular membranes/walls were not noted when proper digestion controls were in place.

All biomass digested was well within industry standard of 20 days, with most achieving 95% CH₄ accumulation prior to day 15. Figure 6.5 shows how the biogas production stabilized well before 20 days when whole cell biomass was digested. CH₄ content ranged from 60–75% while effluent TAN and VFA levels were quite low, indicating effective, complete digestion with little concern of product inhibition, despite all samples having C:N ratios well below that ideally preferred. Throughout all experiments, a certain degree of milling of dried biomass was utilized, thus bringing into question the role the drying and milling processes had in easing AD conditions. This is important as no drying or milling would likely take place in a more realistic commercial environment. Further study on the role of this pretreatment to cellular structures via image processing is warranted.

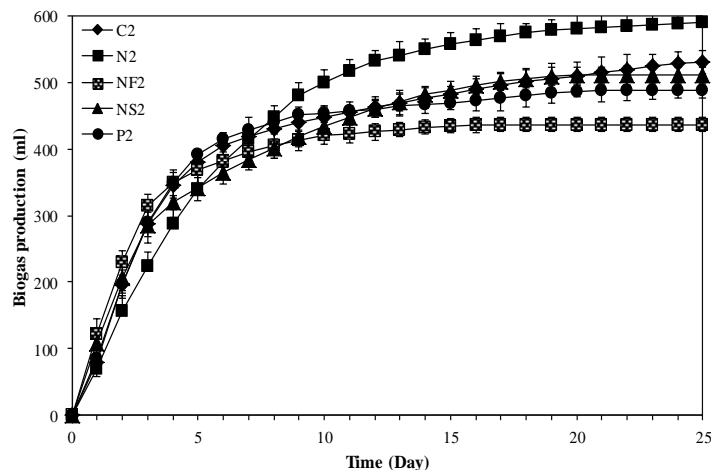


Figure 6.5: Biogas production curves from BMP tests on whole cell biomass

Another observation noted in regard to commercial harvest is the use of extraction solvent mixtures, with studies determining that chloroform/methanol mixtures were extremely inhibitory to methanogenic bacteria (Figure 6.6). Due to this inhibition, it is recommended to experiment with use of different solvent mixtures if AD is to be a critical component of the bio-refinery approach.

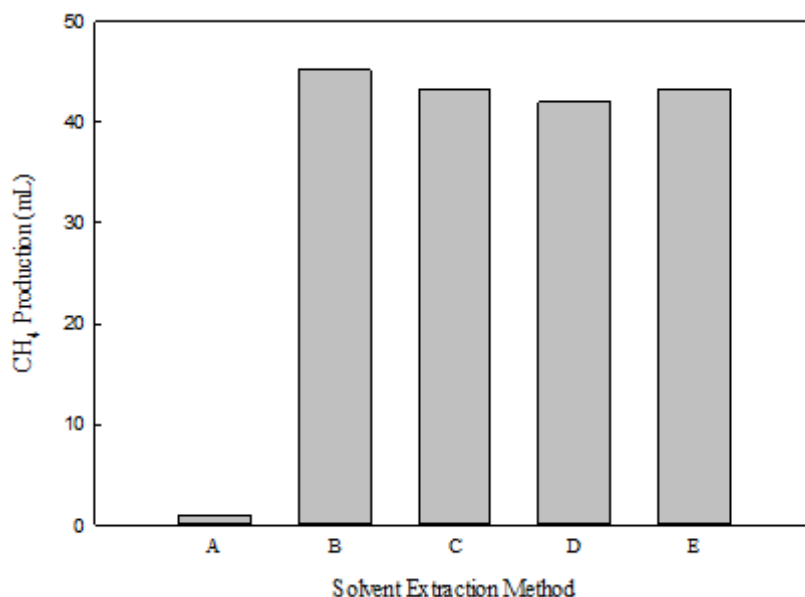


Figure 6.6: CH₄ production during digestion of N1 treated with different solvents A: Chloroform/methanol (2:1); B: Hexane/isopropanol (3:2); C: Dichloroethane/methanol (1:1); D: Dichloroethane/ethanol (1:1); E: Acetone/Dichloroethane (1:1)

Scale-up to continuous digesters showed general maintenance of previously identified BMP capabilities. The NS1 digester showed higher SMP ranging from 0.59–0.65 L CH₄ g VS⁻¹, while the NS2 digester showed a lower SMP ranging from 0.29–0.42 L CH₄ g VS⁻¹. VS reduction percentages at lower OLR were also on par with earlier BMP trials, while percentages decreased at higher OLR tested due to accumulation of undigested algae residue. Results showed that the OLR in the NS2 digester could reach up to 5 g VS L⁻¹ d⁻¹, while the NS1 digester failed at an OLR of 3.0 g VS L⁻¹ d⁻¹. Figure 6.7 illustrates how the OLR affected the biogas production for NS1 and NS2.

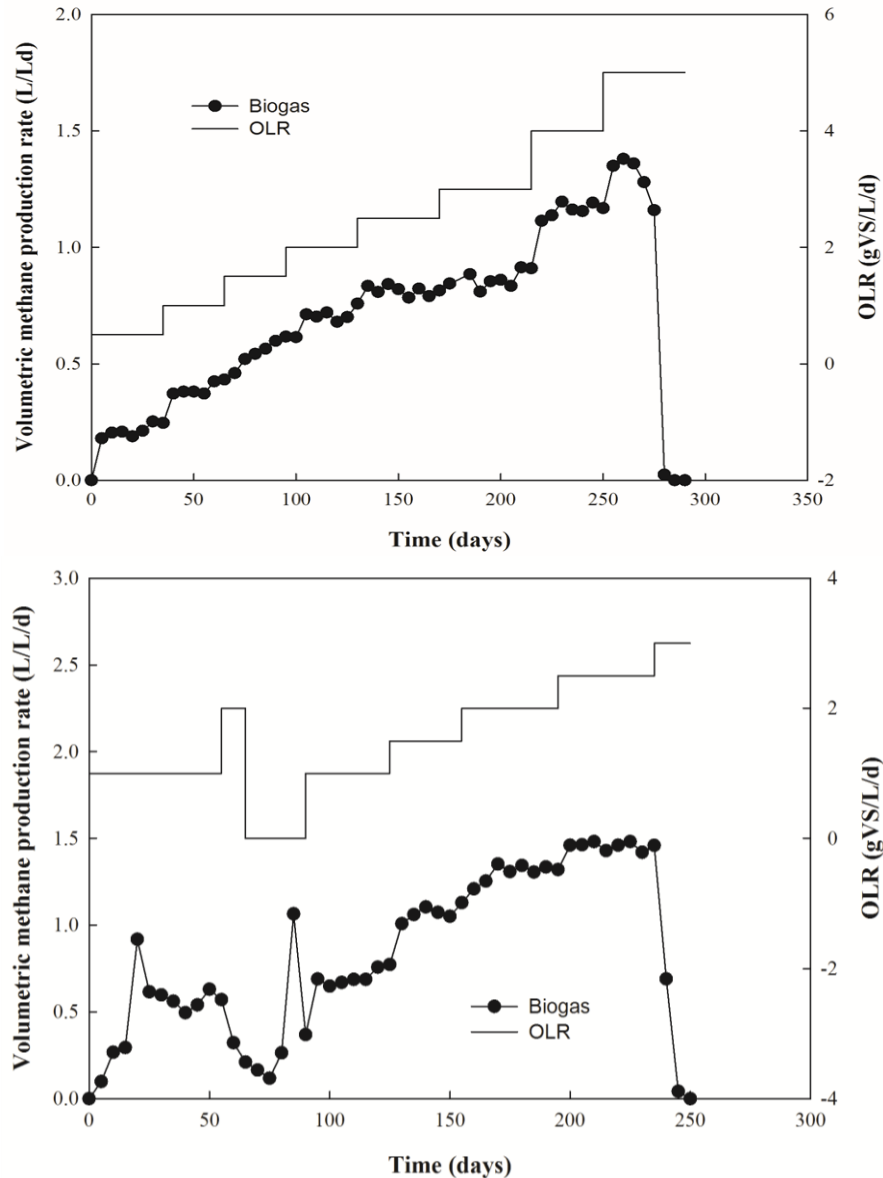


Figure 6.7: CH₄ production from NS1 (*top*) and NS2 (*bottom*) digester at different OLR

Reasons for the decreased viability of NS1 at higher OLR as compared to NS2 is attributed to LCFA inhibition with LCFA accumulating within the digester and attaching to the retained biomass, reducing bacterial performance until ultimately the digester failed. As the ultimate preferred use for AD within the algal bio-refinery concept is to treat lipid-extracted microalgae, the excellent results with NS2 is encouraging, but the noted impact of LCFA on ultimate OLR of NS1 should be useful to those potentially digesting whole cell microalgae.

At these two maximum OLR, both NS1 and NS2 could achieve a volumetric methane potential (VMP) of $1.40 \text{ m}^3 \text{ CH}_4 \text{ m}^{-3} \text{ d}^{-1}$. For comparison purposes, most commercial sludge/manure digesters are deemed effective if they achieve VMP near $1.0 \text{ m}^3 \text{ CH}_4 \text{ m}^{-3} \text{ d}^{-1}$; thus our results

suggest commercial viability. Effluent nutrient concentrations were notably high at higher OLR, allowing for potentially important economic benefits upon recycle of these nutrients to the growth ponds. Effluents and their characteristics were made available to NREL for such recycle/growth studies.

6.3 Conclusions

Microalgae are an attractive renewable energy solution due to their intrinsic ability to accumulate lipids extracellularly, live in harsh environments, and grow at high rates compared to other feedstocks. Most renewable energy research on microalgae has focused on converting extracted lipids into biodiesel. However, the remaining biomass from this process could be utilized by AD to produce electricity or RNG. Therefore, researchers at WSU, working with NREL, tested the biomethane production of five different strains of algae, as either whole cell or lipid extracted. Five different solvent systems were utilized for extracting the lipid from the cell. Based on the studies performed at WSU, the following conclusions were made:

- Chloroform/methanol created sustained inhibitory conditions for AD.
- A linear relationship between SMP and ash-free lipid content was determined, showing the strong relationship that LCFA has on CH₄ production.
- As the I/S ratio increased, so did the biogas production; though the presence of LCFA did have inhibitory effects on the biogas production. This provides further evidence that LCFA should be monitored for successful digestion.
- Dosing the system with Ca raised the level of biogas production.

Anaerobic Digestion Extension-Technology Transfer Project

Craig Frear, Georgine Yorgey, Sylvia Kantor and Chad Kruger

The purpose of the AD Extension-Technology Transfer Project is to provide high-demand educational products and services that reduce technical, financial, and policy barriers to the development of the AD industry. The tasks were developed and prioritized based on an informal needs assessment from industry, government and non-governmental partners. The focus is on assisting industry growth and developing durable extension materials for the industry and industry-serving clientele, rather than (in addition to) scientific publication. The industry commercialization component is summarized in section 7.1 while the durable extension materials are summarized in section 7.2.

7.1 Assisting AD Industry Growth

Several key applied research and extension activities were conducted during this past biennium.

Dr. Frear continues to lead project development, *Pro Forma* analysis and pre-engineering efforts for several projects in Washington that integrate AD of organic waste and NR with a specific aim of producing RNG fuel. Specific projects include:

- **The PacifiClean** project, which is aimed at integrating an AD and NR unit within a planned compost yard. A revised set of Pro Forma and engineering plans were presented last quarter to prospective investors and business developers.
- **Outlook POD**, a large dairy CAFO project located in the Yakima Basin. This project is aimed at responding to area needs in nutrient management and new business models for AD development. Dr. Frear continues to work with project teams to develop the business plans of the project. As of June 2013, the project was entering the financing stage, with plans for construction in spring of 2014.

Much of the outreach work specific to the Yakima Basin project has also been in coordination with the EPA Region 10, with Dr. Frear providing instrumental support in educating/informing both EPA and concerned dairymen during ongoing issues related to nitrate levels in the Basin (EPA, 2013).

In addition to the proposed projects mentioned above, ongoing demonstration of NR research through leveraged funding continues at Lynden and Enumclaw, WA, where two NR demonstration units are being operated alongside anaerobic digesters. Dr. Frear works with industry partners and farmers on a weekly basis regarding attempts to refine and improve the technology. He is also assisting industrial partners in project development across the country.

Beyond work on specific projects, Dr. Frear has continued to explore more sustainable ways to produce the desired co-products, and is working to improve the marketing and usability of developed products. Key tasks that have been implemented include:

- Working with industry and fertilizer distributors to grow mature markets for the developed products from NR (ammonium sulfate soil amendment and P-rich biosolids)
- Working with WSU Prosser scientists in improving knowledge of the effect these bio-fertilizers have, at greenhouse scale, on yield and ecosystem services emissions such as GHG and nitrates
- Coordinating with WSDA officials on registration of fertilizer products and development of allowed storage and containment facilities for the bio-fertilizers.

Lastly, Dr. Frear has made numerous key presentations and met with valuable stakeholders throughout this biennium, all related to development of AD and NR. These presentations include those made at the following events:

- AIChE national conference in Pittsburgh, PA
- USDA NIFA meeting in Washington, DC
- Washington State Bioenergy Symposium in Seattle, WA
- The Far West Agribusiness Association annual conference in Pasco, WA
- EPA AgSTAR national conferences in Boise, ID; Syracuse, NY; and Indianapolis, IN
- Biocycle national conferences in Portland, OR and San Diego, CA
- California Dairy Summit in Modesto, CA
- Center for Dairy Innovation Markets webinar
- ABO Algal national conference in Denver, CO
- Oregon AD Summit in Portland, OR
- EPA AD Markets Summit in Washington, DC
- Qualco Farm Field Day in Monroe, WA
- Washington Dairy Federation meetings in Everett, WA
- US/China AD Symposium in Beijing, China

7.2 Outreach Programs and Materials

7.2.1 Nutrient Recovery Webinar

In October 2011, a webinar entitled “Extracting valuable energy, carbon and nutrient resources from organic waste” was presented as part of the monthly webinar series entitled *WSU Extension: Research that Works for You*. The webinar is described below:

WSU scientists have conducted extensive research on AD as a technology for recovery of CH₄ (energy), stable carbon, and nutrients from organic wastes such as manure, food processing wastes and the organic fraction of municipal solid wastes. Our research has evaluated the technical and economic performance of commercially available systems, developed improved AD reactors, and commercialized WSU patented NR technology (AIR-TRAP system). This potentially game-changing technology could make integrated AD and N and P recovery commercially viable. This webinar, presented by the Center for Sustaining Agriculture and Natural Resources (CSANR)

director Chad Kruger and CSANR scientist Craig Frear, provided an update on the latest results from the WSU Climate Friendly Farming Project's AD research.

The webinar was archived online for asynchronous viewing, and is available at <http://breeze.wsu.edu/p52833652/>.

7.2.2 Anaerobic Digestion Project/Nutrient Recovery System Field Day

A field day/tour of the WSU commercial NR system is scheduled for July 10, 2013. The field day was delayed a few months to allow additional time for final testing of the system to be completed. The planned field day includes visiting the Vander Haak Dairy where the NR system is installed (along with numerous other R&D investments) as well as visiting the newly completed digester installation less than 1 mile away located at Edaleen Dairy. The target audience includes farmers, government agency/regulatory representatives, community and political leaders, AD entrepreneurs/project developers/technology providers, community members, environmentally-oriented undergraduate and graduate students, and Carbon Masters.

7.2.3 Nutrient Recovery Technology Fact Sheet

While working on commercializing AD technology on dairies, it has become increasingly clear that a systems approach that includes NR is essential for comprehensive dairy manure management. WSU has conducted an assessment of the multiple options for recovery of N and P from digested dairy manure. An initial review of this evaluation was presented in chapters 7 and 8 of the Climate Friendly Farming Project Final Report (Kruger et al., 2010). A fact sheet has been drafted that describes the scientific rationale and need for combining NR technology with AD. This fact sheet is being finalized for submission to the WSU Fast Track publication system.

7.2.4 Anaerobic Digestion Project Financial Trade-off Fact Sheet

WSU completed a comprehensive economic analysis of AD systems (Bishop & Shumway, 2009) that included an interactive spreadsheet useful for adjusting scenarios and project costs. This was the most comprehensive economic scenario analysis for dairy AD projects publicly available at the time. However, the data for this study was collected between 2005 and 2006 and the assessment is now dated, given the subsequent upgrades to AD systems and changes in project landscape. Estimates of financial costs and revenues for current AD projects were updated with 2011–2012 figures through a companion project that assessed the feasibility of multi-faceted AD systems installations in eastern and western Washington (Coppedge et al., 2012). This study addressed specific questions that have emerged in recent years, such as financial trade-offs of fabrication materials, relative profitability based on project scale, options for biogas and co-product utilization, and financial trade-offs of auxiliary technology such as NR.

While comprehensive and detailed, this feasibility study is a cumbersome report. Distilling it into a simplified fact sheet will provide up-to-date financial evaluation for commercial AD projects and insight into the conditions that affect project profitability. The primary audiences for this fact sheet are community economic development entities (ports, economic development authorities, and utilities), farmers, consultants and others that need an introduction to the financial concerns associated with digester projects. A secondary audience includes technology providers and project

developers. This fact sheet will be completed and submitted to the WSU Fast Track publication system in June.

7.2.5 Anaerobic Digestion Project Overview Video

Formal and informal evaluations conducted at prior events (workshops, field days, etc.) indicate that most dairy industry clientele in the region have a basic understanding of AD technology and many have personally visited AD projects. However, many of the non-industry clientele that have an influence in project development (i.e. local and state government, financial institutions, utilities, etc.) have not had any kind of formal overview of AD projects and their lack of basic understanding is a barrier to the development of specific projects. An overview video, with an emphasis on providing visuals, was deemed the most effective and efficient media for providing a succinct overview of AD systems, reducing the need and expense of hosting future AD 101 workshops. The overview video has been finalized and posted on a permanent YouTube Channel: <http://youtu.be/Ei49Z4oeUtY>. In cooperation with Marketing and News at WSU's College of Agriculture, Human, and Natural Resource Sciences, we publicly "released" the video on June 10, 2013 as part of the promotional campaign for the July field day.

7.2.6 Anaerobic Digestion Workshop at Bioenergy Research Symposium

The state's Bioenergy Research Symposium has become the most effective venue for sharing the rapid advances in AD research in the region. We hosted a workshop session for the 2012 Symposium. The session is described below:

Innovations in Bioprocessing

Increasing interest in waste-to-energy technologies, nutrient management, process efficiencies and economies-of-scale is driving innovative research into bioprocessing pathways. This panel will explore exciting discoveries looking to advance to commercial-scale applications.

Moderator: Craig Frear, WSU

- NR from High-Solids AD (Craig Frear, WSU)
- New High-Solids AD Systems (Shulin Chen, WSU)
- Integrating Pyrolysis and AD (Manuel Garcia-Perez/Matthew Smith, WSU)
- Breaking the Barrier of Biomass Conversion Using Wet Explosion Pretreatment (Philip Teller, WSU)

The session presentations were archived online for asynchronous viewing, and are available at <http://www.pacificbiomass.org/BioenergyResearchSymposiums/BioenergyResearchSymposium2012.aspx>

Smaller Projects on Various Anaerobic Digestion Topics

8.1 Psychrophilic Anaerobic Digestion

Jingwei Ma, Jie Liu and Shulin Chen

8.1.1 Introduction

Throughout the Northern Hemisphere, numerous household and commercial AD facilities have been constructed. However, a majority of these facilities have either ceased functioning or yield low amounts of biogas due to poor construction, lack of awareness, improper feeding, and souring during winter. For example, a biogas facility located in the Himalayas had 70% lower biogas output during the winter season than in the summer (Kanwar & Guleri, 1994). This is undesirable from an economic standpoint but also, more importantly, from an energy standpoint – as it means AD is least able to meet demand during the winter when demand is at its peak. This is a serious concern when biogas plants are used for cooking and heating, forcing the users to seek other fuels to replace this loss of energy (Kashyap et al., 2003). Winter souring is the result of an inability to maintain an effective heat and temperature balance within the digester. This can occur for one or more of the following reasons:

1. Improper engineering to adequately overcome heat losses in cold environments
2. Over-dilution
3. Cold wastewater
4. Economic factors which prevent the inclusion of heating or heat recovery systems
5. Anaerobic microorganism populations that are negatively affected by a drop in temperature

At lower temperatures, the mesophilic bacteria that dominate at higher temperatures (32-38°C) are unable to sequester substrates from their environment because of lowered affinity (Nedwell, 1999). This results in lower biogas production compared to digesters operating at the higher temperatures that mesophilic bacteria thrive in (Kashyap et al., 2003). One proposed method for overcoming this lower degradation is slowing down the loading rate and increasing the HRT to over 100-300 days (Safley & Westerman, 1990). However, this would require larger digesters to handle the higher volume of organic matter and is not viable for household digestion. An alternative solution is to find other ways to improve the biogas production process without substantially altering the digester itself.

One approach to overcoming this phenomenon is to utilize psychrophilic microbes, since they are more adept at surviving, and even thriving in, low temperature environments. The Arrhenius equation has been used to show how the losses in biogas production occur at lower temperatures through a correlation between the active temperature ranges of anaerobic microorganisms and their subsequent biogas production (Schütz et al., 1990). However, there still are benefits to such design as reduced but stable performance during winter is preferred over complete stoppage or sustained, extensive inhibition. Methanogenesis has been reported in diverse cold habitats including arctic

and sub-arctic peat lands (Metje & Frenzel, 2007), freshwater sediments (Nozhevnikova et al., 2001), and high-altitude rice paddy soils (Fey & Conrad, 2000).

Several psychro-active acetogenic bacteria and psychro-active methanogenic archaea have been isolated from cold environments and have shown growth potential at temperatures below 10°C (Nozhevnikova et al., 2001); however, the microbial interactions and biochemical pathways involved are still not well understood (Kashyap et al., 2003). Studies have been conducted on low temperature digestion but were later found to be psychrotrophic bacteria, which are more capable of handling temperature changes than psychrophiles (Kashyap et al., 2003). Due to these misunderstandings, the most adept microorganisms have yet to be extensively studied for their effectiveness at producing biogas at low temperature. Therefore, research on psychrophilic biomethanation was carried out as part of an effort to develop a new generation of anaerobic fermentation technologies that can overcome the biogas reductions currently seen at digesters in regions with extreme temperature swings.

8.1.2 Biogas Production from Low Temperature Sources

Samples from low temperature environments were screened for psychrophilic consortia at WSU. Ten samples of microbial consortia from different environments were chosen to enrich psychrophilic microorganisms or psychro-active microorganisms. The ten samples originated from the following locations: three separate lagoon locations located at WSU Knott Dairy Center (L₁, L₂, and L₃), a mix tank located at WSU Knott Dairy Center (Mix), a lab-scale sequential batch reactor fed with flushed dairy manure located at WSU (SBR), waste-activated sludge from the Pullman Wastewater Treatment Plant (WWTP) located in Pullman, WA (WAS), an aeration tank from the PWTP located in Pullman, WA (Aeration), a household digester located in LD, Heilongjiang, PRC (LD), a household digester located in SH, Heilongjiang, PRC (SH), and a household digester located in XW, Heilongjiang, PRC (XW). All of these locations undergo significant temperature swings from summer to winter and, because of this, should be appropriate locations for discovering psychrophilic bacteria.

8.1.2.1 Results and discussion

Table 8.1 depicts the different locations of the inoculums subdivided into three categories including dairy lagoon, digester and WWTP. Dairy manure was used as substrate for biogas production. Fresh dairy manure with TS of 180.7 g/L and VS of 151.3 g/L was collected from the WSU Knott Dairy Center in Pullman, WA and stored at 4°C prior to use.

Table 8.1: Biogas potential generated from different microbial consortia

Category	Dairy lagoon				Digester				WWTP	
Inocula source	L ₁	L ₂	L ₃	Mix	SBR	LD	SH	XW	WAS	Aeration
Biogas (mL)	2523	2326	2355	2817	1911	2020	2277	1877	1562	1251

Experiments were conducted in 250 ml serum bottles that were kept inside a temperature-controlled room at 15°C. Biogas production was monitored daily and gas content was analyzed by

gas chromatography (GC). Each set was run in triplicate to ensure reproducibility of results. The biogas production profile is shown in the Figure 8.1 and compared in Table 8.1.

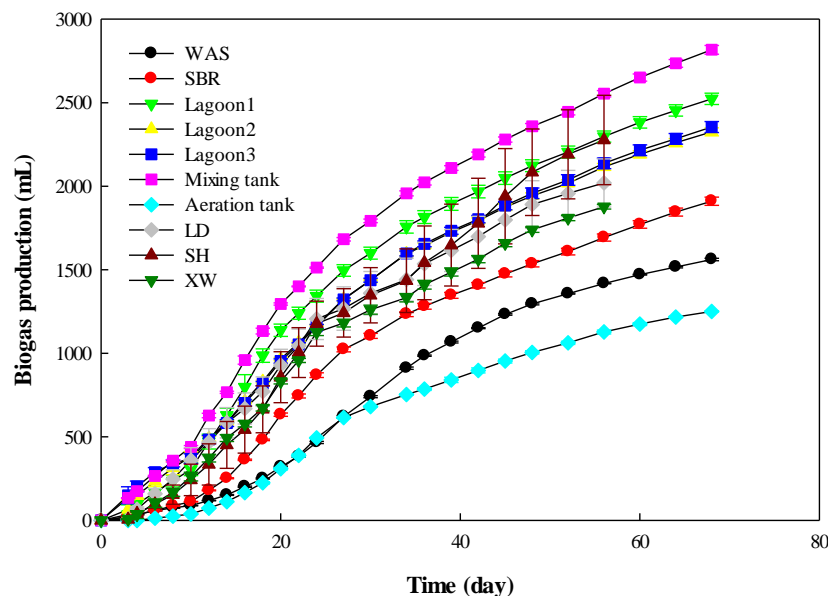


Figure 8.1: Biogas production from 10 low temperature consortia (triplicate)

Anaerobic microorganisms from dairy lagoons and digesters showed a higher biogas production rate than aerobic microorganisms from active sludge from the WWTP as they adapted to anaerobic environment better than aerobes. Biogas production from digesters inoculated with dairy lagoon (L_1 , L_2 , and L_3) microorganisms presented a high biogas production rate due to undigested manure in the seeds. Taking this into account, the microbial consortia sampled from the long-running digesters, especially SH and LD, were considered to be good candidates for psychrophilic consortia.

8.1.2.2 Conclusions

Psychrophilic consortia from long-running digesters showed good biogas production. However, psychrophilic consortia from dairy lagoons showed the highest biogas production since undigested dairy manure was associated with inoculum. If we take this unforeseen occurrence into consideration, the psychrophilic consortia from SH digester, which didn't have undigested dairy manure in the seed, resulted in the highest biogas production. Therefore, the psychrophilic consortia inhabiting the SH inoculum will be used for further analysis and the performance will be compared with mesophilic AD. The studies performed provided initial data on discovering the best fit consortia for further analysis. The next step in this process will be to generate low-temperature anaerobic digestion (LTAD) technology that will utilize the psychro-active consortia with the goal of developing high treatment efficiency, low construction and operation cost digestion technology to produce multiple products using real-time, object-oriented control systems.

8.1.3 Enhancing Biogas Production of Psychrophilic Anaerobic Digestion with Trace Elements

Bacteria require macronutrients and trace elements such as N, P, sulfur (S), K, Ca, magnesium (Mg), Fe, nickel (Ni), cobalt (Co), zinc (Zn), manganese (Mn), and copper (Cu) to grow optimally (Rajeshwari et al., 2000). Unfortunately, wastewater streams can lack the required concentrations of some of these micronutrients and trace elements, requiring supplementation prior to digestion (Amani et al., 2010). Since trace elements play such an important role in AD, studies were performed at WSU on the effect trace elements have on the psychrophilic bacteria. Two psychrophilic consortia (SH and LD) from long-running digesters with higher CH₄ production were selected as target consortia in this research for evaluation of the effects of adding trace elements on biogas production. The TS and VS of SH were 16.0 g/L and 12.6 g/L, respectively. TS and VS of LD were 22.7 g/L and 18.2 g/L, respectively. Dairy manure was used as substrate for biogas production. Fresh dairy manure with TS of 180.7 g/L and VS of 151.3 g/L was collected from the WSU Knott Dairy Center in Pullman, WA and stored at 4°C prior to use.

During BMP assays, 5 g VS of dairy manure were inoculated with 60 mL of psychrophilic seeds and placed into 250 ml flasks that served as bioreactors for the BMP studies. The working volume in each BMP reactor was 200 ml with the rest serving as headspace. Optimal trace elements concentration for CH₄ production was determined in a previous study to be 1 mg/L of Fe, 0.25 mg/L of Ni, and 0.2 mg/L of Co. Digesters without trace elements addition served as the control. All digesters were incubated in a low temperature chamber at 15°C. Biogas production was monitored daily and gas content was analyzed by GC. Each set was run in triplicate to ensure reproducibility of results.

8.1.3.1 Results and discussion

Figure 8.2 shows the accumulated biogas production of digesters inoculated with SH (left) and LD (right). With the trace elements addition, the biogas and CH₄ production from SH increased by 28% and 17%, respectively. However, no notable enhancement or inhibition was observed in biogas production of LD digester. This indicates that the demand for trace elements is species specific for psychrophilic consortia. When compared with a typical mesophilic digestion process (0.23 L CH₄ gVS⁻¹), the ultimate CH₄ yield under psychrophilic conditions (0.14 L CH₄ gVS⁻¹) was quite low, even after trace element addition. However, without input energy for heating, the psychrophilic digester may still be able to compete with the mesophilic digester in terms of net energy output. A techno-economic analysis is required to further evaluate this possibility.

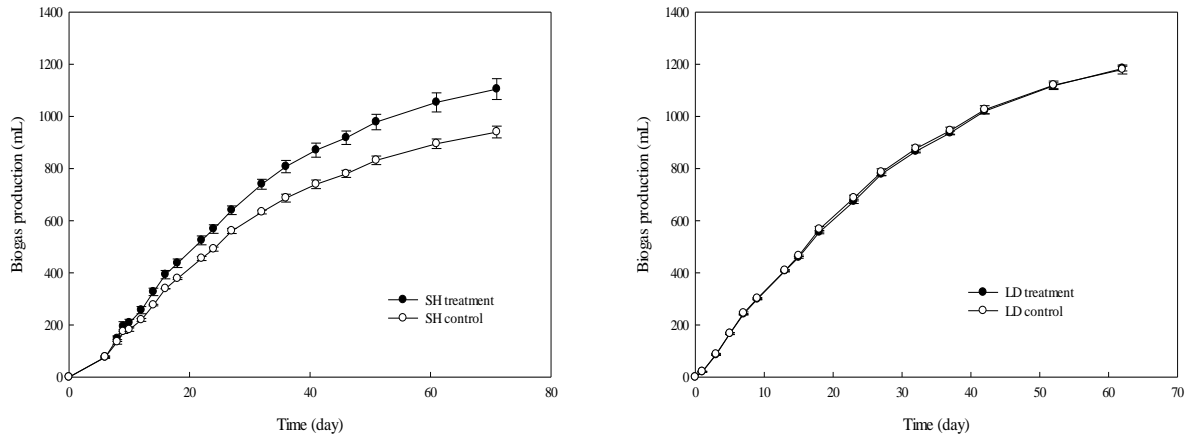


Figure 8.2: Biogas production of SH inoculated digester (*left*) and LD inoculated digester (*right*)

8.1.3.2 Conclusions

Our studies showed that low temperature digestion is possible and that anaerobic bacteria in certain locations are better adapted to low temperature conditions. Our results suggest that when inoculating an anaerobic digester in an area subject to wide temperature swings, it is advantageous to choose a substrate from a digester that has operated at low temperatures for an extended period of time. To fully justify the claim that the best-performing SH consortia was comprised of psychrophilic bacteria, a clone library should be constructed and used to identify the type of methanogens present.

Psychrophilic microbes require a higher concentration of trace elements than mesophilic microbes. However, the demand for trace elements is species specific with different bacteria or archaea requiring different trace elements for enhanced digestion. Therefore, this strategy may need to be optimized for each digester.

Since many dairy farms are operated in the Northern regions of the U.S., China, and Europe, psychrophilic consortia may prove beneficial for both waste management and bioenergy production, particularly in cases where heating requirements for mesophilic digestion cannot be maintained.

8.2 Carboxylate Platforms

Baisuo Zhao, Craig Frear and Shulin Chen

8.2.1 Introduction

As discussed more fully in Chapter 6, research on the use of microalgae for biodiesel and for AD have both recently received increased attention. Within the topic of AD of microalgae, the low C:N ratio from algal biomass is a serious problem since algae biomass typically has high protein content (39–71%), which could produce high TAN and result in the inhibition of methanogenesis (Sialve et al., 2009). One solution that has been implemented by other researchers is co-digestion of microalgal biomass with another substrate that is high in C and low in N. This can diminish ammonia toxicity and increase biogas production. For example, biomethane yield and productivity doubled when equal masses of wastewater sludge and *Spirulina* biomass were co-digested (Samson & Leduyt, 1982). Similarly, Yen and Brune (2007) added waste paper (50% w/w) to aquaculture algal sludge to adjust the C:N ratio to around 20–25:1 which, in turn, doubled the CH₄ production rate from 0.6 L/L liquid day to 1.2 L/L liquid day at 35°C with a HRT of 10 days.

Recently, another advanced biorefining technology, the MixAlco process within the ‘carboxylate platform,’ was developed by Holtzapple and colleagues (Agler et al., 2011; Holtzapple et al., 1999; Holtzapple & Granda, 2009). This technology can convert complex waste into mixed carboxylate salts, an effective feedstock for downstream production of biofuels and valuable targeted chemicals, using an undefined, anaerobic acid-forming bacterial consortia. In theory, the high concentrations of TAN from algal protein during the fermentation are easily acidified, resulting in the accumulation of high concentrations of carboxylic acids. Simultaneously, the high concentration of NH₄⁺ derived from algal TAN is able to neutralize the produced carboxylic acids, which can directly lead to a low supplement salt bicarbonate buffer (Fu & Holtzapple, 2010b). The cow rumen contains more than 1,000 bacterial OTUs associated with the biodegradation of lignocellulose (Hess et al., 2011). These bacteria provide rapid fermentation rates from the complex feedstock with high productivity of short-chain volatile carboxylic acids, including acetic, propionic, and butyric acids (Weimer et al., 2009).

The goal of this work was to establish a mixed culture of cow rumen bacteria for the transformation of microalgal biomass to carboxylates using ASBR technology as a consolidated bioprocessing (CBP) system. Figure 8.3 illustrates this consolidated process to produce carboxylate with subsequent NR and downstream processing to biofuels.

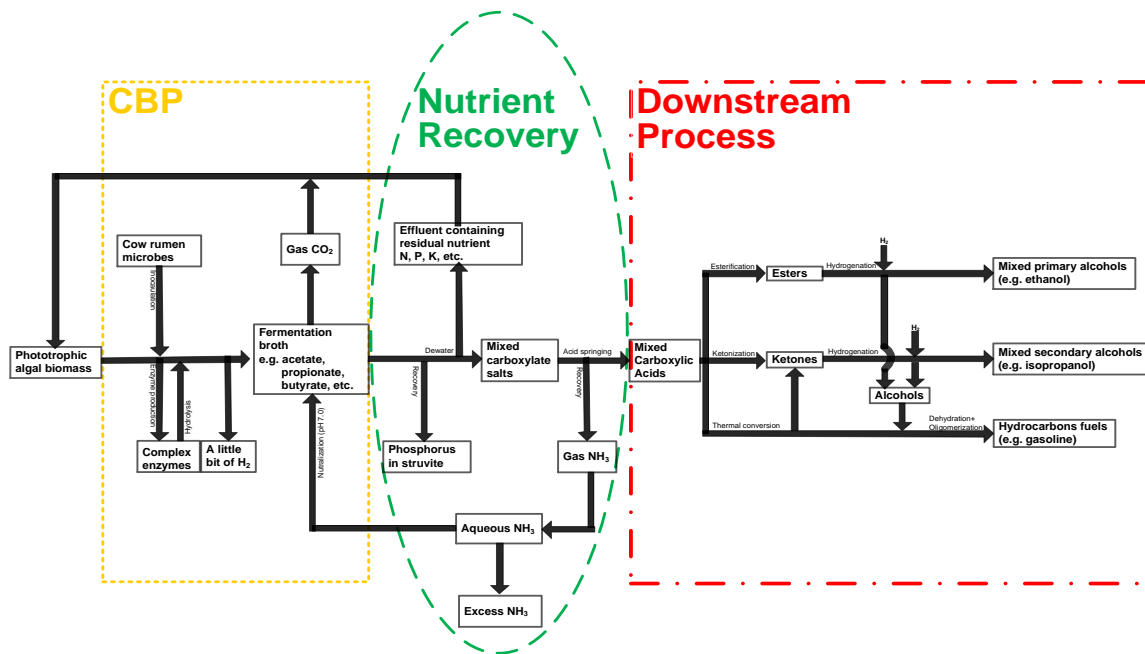


Figure 8.3: Overview of the CBP from algal biomass to carboxylate salts

During the experiments, the bacterial population dynamics and community were investigated with T-RFLP technology. Understanding the microbial population and its community structure will help us to (1) refine the design and operation of the bioreactor system, (2) facilitate the development of inoculum for large-scale implementation of this process, and (3) contribute to the developing body of knowledge regarding conversion and biofuel production from algal biomass.

8.2.2 Methods

8.2.2.1 Feedstock and inoculum source

Scenedesmus dimorphus, a freshwater unicellular alga of class *Chlorophyceae*, was used in this study as the biomass feedstock for a series of fermentation experiments. The media for *S. dimorphus* cell culture was composed of 5 ml feed concentrate per gallon filtered tap water. *S. dimorphus* were photoautotrophically cultured for 15 days at pH 6.0–8.4 at a temperature of 22–25°C in a closed pond. The algal biomass of *S. dimorphus* was harvested, dewatered, and dried using the HDD technology described by Algaeventure Systems (<http://www.algaevs.com/>). The seed inoculum of the mixed fiber and liquid from cow rumen used in the ASBR reactor was obtained from the animal science farm at WSU in November 2010.

8.2.2.2 Bioreactor construction and performance monitoring

4 g dried algal biomass of *S. dimorphus* and 10% seed inoculum were added to four serum bottles for the ASBR. The initial volatile solids loading rate (VSLR) was decreased stepwise by increasing HRT from 4.2, 8, 12, and 16 days. The experimental design conditions for the different HRT in the ASBR system are shown in Table 8.2.

Table 8.2: Operational parameters and characteristics of un-extracted algal biomass samples

ASBR fermentation	A	B	C	D
HRT (days)	4.2	8	12	16
VSLR (g VS fed/L liquid day)	7.51	3.96	2.65	1.98
Total carboxylic acid concentration (g/L liquid)	3.62±0.63	7.50±0.81	12.35±0.53	14.06±0.59
Product distribution				
Acetic acid (wt %)	54.85±7.96	49.16±9.88	44.90±3.33	36.28±3.50
Propionic acid (wt %)	15.48±2.76	15.23±1.83	14.56±1.52	12.21±1.69
Butyric acid (wt %)	19.23±1.41	22.04±1.94	21.48±0.75	21.48±2.17
Valeric acid (wt %)	10.43±1.35	13.57±0.82	13.88±1.49	17.12±3.29
Caproic acid (wt %)	0.00±0.00	0.00±0.00	4.18±2.91	6.72±1.39
Heptanoic acid (wt %)	0.00±0.00	0.00±0.00	0.00±0.00	6.58±0.68
Conversion (g VS digested/g VS fed)	0.208	0.381	0.526	0.598
Yield (g total acids/g VS fed)	0.115	0.237	0.388	0.443
Selectivity (g total acids/g VS digested)	0.552	0.621	0.738	0.742
Total carboxylic acid productivity (g total acids/L liquid day)	0.862	0.938	1.029	0.879
Acetic acid yield (g acetic acid/g VS fed)	0.063	0.116	0.174	0.161
Methane productivity (g CH ₄ /L liquid day)	0.0002	0.0009	0.0006	0.0010
TAN (mg/L)	522.2	551.7	584.7	626.7

The lab-scale ASBR bioreactor (New Brunswick Scientific Inc., Model Bioflo 110 Fermentor, NJ) was operated with 5 L working volume supplied with 3% seeds of 12 HRT (Figure 8.4) at 39±1°C and 200 rpm.

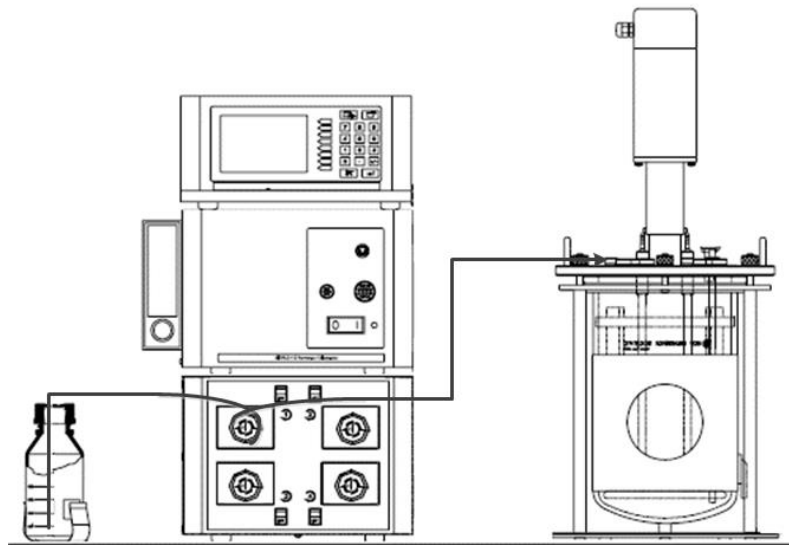


Figure 8.4: Schematic of the fermentation process with attached monitoring system

Methanogens were inhibited by a concentration of 25 mg iodoform/L which came from a concentrated solution (25 g iodoform/L ethanol) during the fermentation (Fu & Holtzapple, 2010a). The concentrated solution was stored in a sterile sealed N₂-full serum bottle encased in aluminum foil at 4°C due to the sensitivity of iodoform to light and air. The iodoform was taken through the butyl rubber stopper with the 1 ml syringe, and added into the reactor every two days to prevent CH₄ production.

8.2.3 Results and Discussion

8.2.3.1 Characteristics of *S. dimorphus* biomass

The biomass of *S. dimorphus* was harvested at approximately 0.5 g dry weight/L liquid with 0.791 g VS/g TS and 0.291 g Ash/g TS, implying this strain can offer abundant metal elements for microbial nutrition during anaerobic processes. The significant organic nutrients were C (39.62%), H (5.66%), O (28.66%), N (6.65%), and S (1.25%), respectively. The crude protein is generally regarded to be 6.25 times total N, which indicates that the protein content of *S. dimorphus* was approximately 41.6%. This is consistent with the protein range of values (29–71%) given by Sialve et al. (2009). The total lipids were only 7.22% because it was grown phototrophically without any nutrient stress to induce lipid accumulation. Three prominent fatty acids were present including palmitic (C_{16:0}, 13.75 %), linoleic (C_{18:2n6}, 8.60 %), and linolenic (C_{18:3n3}, 7.63 %), respectively. The total lipid content in *S. dimorphus* was less than 40%. Generally, a C:N ratio of 20–30:1 is considered optimal for the production of CH₄, though there is still some disagreement (Parkin & Owen, 1986; Yen & Brune, 2007). The ratio of C:N in *S. dimorphus* was 5.96/1, too low to generate CH₄, but could be used to generate carboxylates.

8.2.3.2 Carboxylic acids production in anaerobic sequence batch reactor with different hydraulic retention times

Continuous carboxylic acid production was operated simultaneously in ASBR with 100 ml working volume at different HRT including 4.2 (A), 8 (B), 12 (C), and 16 (D) days. As HRT increased, the VSLR decreased to 7.51, 3.96, 2.65, 1.98 g VS fed/L liquid day, respectively. Four reactors were running for 80 days with *S. dimorphus* as a feedstock. Steady-state production of the total carboxylic acids was achieved during days 60–80 (data not shown). Table 8.2 summarizes the operating parameters and constant experimental results of four HRT fermentations. Data shows that production was impacted by HRT, with resulting variation in the total carboxylic acid concentration, productivity, yield, conversion, selectivity, and even metabolite patterns. With 4-day and 8-day HRT, low digestibility occurred although the VSLR was high. The main reason for this is that the algal cell wall is composed of a diverse array of fibrillar, matrix and crystalline polymers, which are not easily biodegradable by bacteria in a relatively short operational period. With 12-day and 16-day HRT, the digestibility was significantly enhanced, indicating that specific microbial consortia selected under pressure of HRT have been established for the biodegradation.

The maximum total carboxylic acid yield (0.879 g/L day), selectivity (0.742 g total acids/g VS digested) and conversion (0.598 g VS digested/g VS fed) occurred at 16-day HRT fermentation (VSLR = 1.98 g VS fed/L liquid day) with a total carboxylic acids concentration of 14.06±0.59 g/L liquid. In contrast, the highest productivity (1.029 g total acids/L liquid day) resulted from the 12-day HRT fermentation (see Figure 8.5) (VSLR = 2.65 g VS fed/L liquid day) with a concentration of 12.35±0.53 g/L liquid. Since productivity of total carboxylic acids is preferred by industry, this suggests that the optimum HRT was 12 days. The profiles and proportions of individual acid from *S. dimorphus* biomass in the effluents were significantly different from those of sugarcane bagasse and sewage sludge (Fu & Holtzapple, 2010a; Fu & Holtzapple, 2011; Rughoonundun et al., 2010). As indicated in Table 8.2, the patterns and concentrations of carboxylic acids shifted from lower molecular weight acids (i.e. acetic acid and propionic acid) to higher molecular weight acids (i.e. valeric acid, caproic acid and heptanoic acid) with an increase in HRT. For instance, the major carboxylic acids from *S. dimorphus* consisted of acetic acid

(36.28–54.85 wt % of the total carboxylic acids), propionic acid (12.21–15.48 wt %), butyric acid (19.23–22.04 wt %) and valeric acid (10.43–17.12 wt %).

In order to effectively test and enhance total carboxylic acid productivity for a modest scale-up at an HRT of 12 days, a 5 L working volume lab-scale ASBR bioreactor was operated under conditions designed to mimic common industrial operational conditions. After 32 days of incubation, the performance metrics were quite stable and significantly greater at the 5 L working volume than at the 100 ml working volume. The maximum total carboxylic acid concentration of 14.68 ± 0.31 g/L liquid was reached after 20 days (see Figure 8.5) and the yield of 0.462 g total acids/g VS fed was achieved, higher than yields from the previous study. Total carboxylic acid productivity was 1.223 g total acids/L liquid day with a biomass conversion of 63.15% g VS digested/g VS fed and selectivity of 0.731 g total acids/g VS digested.

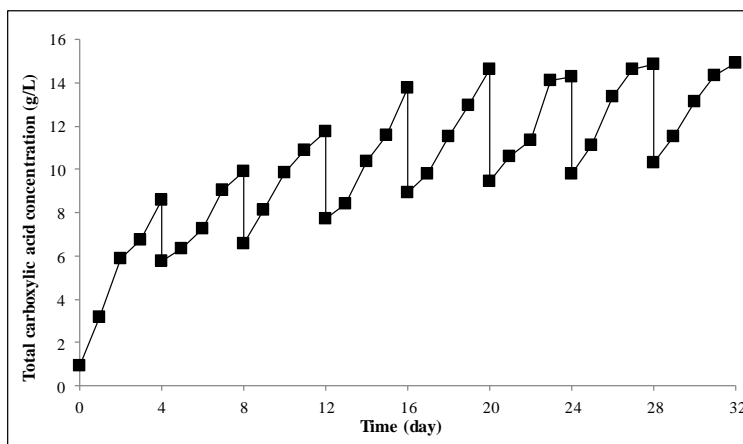


Figure 8.5: The total acid concentration for the lab-scale ASBR bioreactor at a 12-day HRT

During anaerobic fermentation, protein is degraded and the accumulated NH_4^+ (the main form of N) can be released into liquid phase (Zamalloa et al., 2011). The TAN of liquid effluent was on the average 1.165 g/L, which accounted for approximately 84.4% of the mixed solid and liquid effluent. This data indicated a lower concentration than has been found from the cyanobacteria *Spirulina maxima* (up to 7.0 g/L) (Samson & LeDuyt, 1986) and higher than has been found from marine microalgae *Phaeodactylum tricornutum* (0.546 g/L) (Zamalloa et al., 2011). The strong concentration of ammonia from *S. dimorphus* also suggested the protein rich microalgae are unable to produce a high volume of biomethane as ammonia has some toxic effects on methanogenesis. As Table 8.2 shows, the ammonia can be recovered from the digestion effluent using sulfuric acid as an adsorbent through aeration at elevated temperatures of 50°C (data not shown). The soluble phosphates ($\text{PO}_4\text{-P}$) in the effluent from *S. dimorphus* was on average 0.267 g/L, whereas total phosphate was on the average of 1.898 g/L, indicating that there are still almost 86% phosphates in the solid effluent. These data indicate that recycling N and P from algal waste via the ‘carboxylate platform’ is a promising way to address the nutrient needs of microalgae cultivation.

8.2.3.3 Terminal restriction fragment length polymorphism analysis of bacterial community at different hydraulic retention time

The T-RFLP profiles of bacterial fragments digested with Hae III indicated that the percentages of each T-RF varied significantly in the four reactors, suggesting dynamic shifts had occurred in the bacterial communities. One major T-RF, 274, could be seen at HRT 4 days but disappeared at HRT 8, 12, and 16 days. There were several major T-RF of 112, 175, 339, and 340 bp when 8, 12, and 16 HRT were utilized, corresponding to the dominant bacterial (OTU) (Figure 8.6). However, these T-RFs were not dominant at the HRT of 4 days. Results such as these indicate a bacterial shift at higher HRT. They also indicate that monitoring with T-RFLP may indicate changes in bacterial communities when changing different parameters. Improved understanding of these shifts in response to operational parameters could allow researchers in the future to promote desired bacterial shifts to generate more biogas or other co-products.

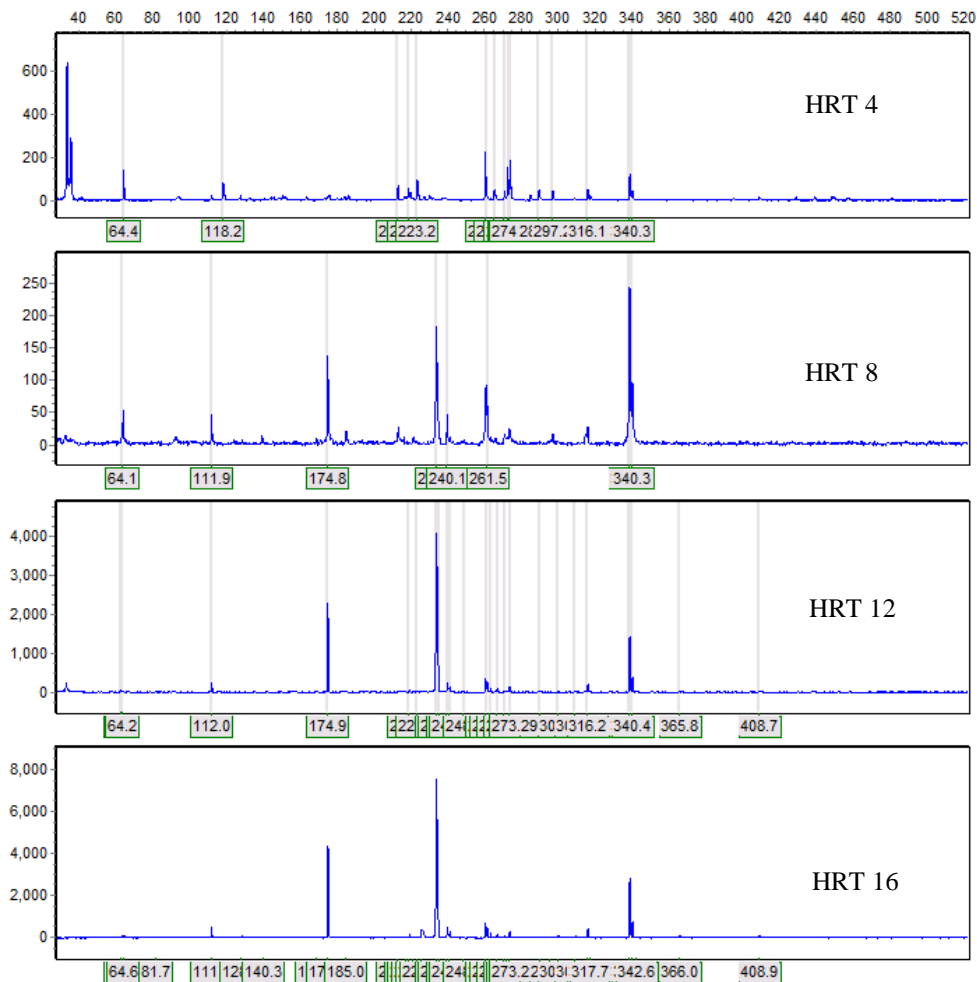


Figure 8.6: T-RFLP pattern of PCR-amplified nearly full-length bacterial 16S rRNA genes of different HRTs digested with the restriction enzyme Hae III

8.2.4 Conclusions

S. dimorphus was investigated in this study for its ability to produce carboxylate salts, which can be further processed downstream to carboxylic acid and ultimately biofuels (e.g. ethanol, isopropanol, or gasoline). The experiments conducted in this project showed that the production of carboxylates was impacted by the HRT, with the highest production occurring at an HRT of 12 days. Thereafter, *S. dimorphus* was anaerobically digested in a 5 L working volume ASBR reactor to showcase the production of carboxylate acids under operational conditions that more closely represented those found in industry. A maximum total carboxylic acid concentration of 14.68 ± 0.31 g/L liquid was achieved after 20 days and thereafter steady state production was achieved. The pathway to carboxylate salts provides another option for the AD of algal biomass residue, one that may be more appropriate than the production of biomethane. Analysis with T-RFLP indicated that the community shifted over time during the digestion process, and a clone library of the species at the 12 day HRT is currently being constructed to provide more insight into the dominant bacterial community.

8.3 Methane Super-Saturation

Quanbao Zhao, Changle Pang and Craig Frear

8.3.1 Introduction

In addition to generating CH_4 and CO_2 (Fine & Hadas, 2012; Massé & Droste, 2000), AD discharges a large volume of liquid effluent which contains dissolved gases, including CH_4 , CO_2 , and H_2S (Hudson et al., 2006; Sharpe et al., 2002; Ullman & Mukhtar, 2007). In the past, the dissolved methane (D- CH_4) in effluent has gone unrecovered (Hatamoto et al., 2011; Hatamoto et al., 2010) and likely escapes during lagoon storage and/or field application. Emitting these gases into air pollutes the environment and contributes to climate change as CH_4 is a powerful GHG (Abbasi et al., 2012; VanderZaag et al., 2011). In addition, the D- CH_4 that is discharged with effluent represents a loss of energy that could have been used to generate energy.

Some authors have reported that D- CH_4 in the effluent can range from 36–41% (Souza et al., 2011), to 50% (Agrawal et al., 1997), to more than 60% (Singh & Viraraghavan, 1998) of the theoretical CH_4 potential in the reactors. Souza et al. (2011) and Hartley and Lant (2006) indicated that D- CH_4 was super-saturated in the effluent of many anaerobic treatment systems. Conditions of low temperature and low strength lead to D- CH_4 , as the solubility of CH_4 in the liquid phase increases with decreasing temperature. Since most digesters operate at mesophilic temperature ($\sim 35^\circ\text{C}$), a portion of the CH_4 generated by methanogenic microorganisms remains within the effluent. Due to the economic and environmental implications of D- CH_4 , researchers at WSU developed a technique to extract the D- CH_4 within dairy effluent.

8.3.2 Methods

A continuous stirred-tank reactor (CSTR) reactor with a working volume of 1 L was used to perform the experiments. Attached to the CSTR were a temperature detector, heater and agitator. A vacuum pump (N816.3KT.45P, KNF, USA) and gas collector were connected to the reactor and

used to determine the concentration of CH_4 , CO_2 , and H_2S . A schematic of the system is shown in Figure 8.7.

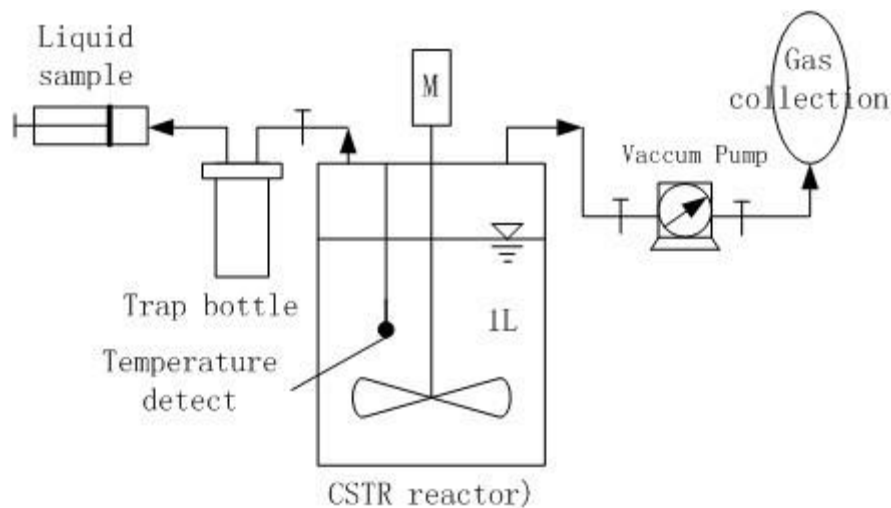


Figure 8.7: CSTR reactor with attached vacuum pump and gas collection system

The reactor was fed with 1 L volume dairy AD effluent from a dairy farm. The initial pH of the effluent was 7.9 ± 0.08 . About 1 ml defoaming solution was added into the effluent to control foam production during the experiment. The CSTR reactor was operated at different temperatures and mixing speeds to investigate the effects these two parameters had on removing D- CH_4 , dissolved CO_2 (D- CO_2), and dissolved H_2S (D- H_2S). The operational conditions are summarized in Table 8.3.

Table 8.3: Operational conditions of the experiments

Parameter	Unit	Values
Temperature	$^{\circ}\text{C}$	25, 35, 55
Mixing speed	rpm	0, 300

Three sealed vials of known working volume (250 ml) were used to sample 200 ml effluent with 110 ml volume headspace and thereafter 1 ml of 20 mM mercury (II) chloride was added to each vial to inhibit biological reactions. The vials were shaken vigorously on a shaker at ambient temperature for one hour to allow the dissolved gas in the effluent to diffuse into the headspace. After equilibration of the gas and liquid phases, the compositions of the headspace gas were determined by GC (Varian CP-3800, Agilent, USA).

8.3.3 Results and Discussion

The D- CH_4 recovery performance was highly dependent on the temperature and mixing speed. Figure 8.8 depicts the cumulative recovery results of the D- CH_4 in the effluent at different temperatures and mixing speeds. From the results of the vacuum recovery, it is clear that for 1 L of effluent, the cumulative D- CH_4 recovery sharply increased in the first 5 mins and continued increasing, though the recovery rate gradually slowed after about 15 mins under most conditions. This can be attributed to the fact that the initial concentration of the D- CH_4 was relatively high in the effluent and low in the headspace of the reactor; thus, the gas-liquid equilibrium was relatively

easy to overcome under vacuum conditions. As D-CH₄ was removed over time it became more difficult for the vacuum to remove D-CH₄. Under some experimental conditions, equilibrium took longer to achieve. For example, at 25°C and 0 rpm the equilibrium wasn't achieved until around 60 mins. In addition, the total recovery was much lower. Clearly, the combination of low temperature and low rpm results in a much lower release of dissolved gases.

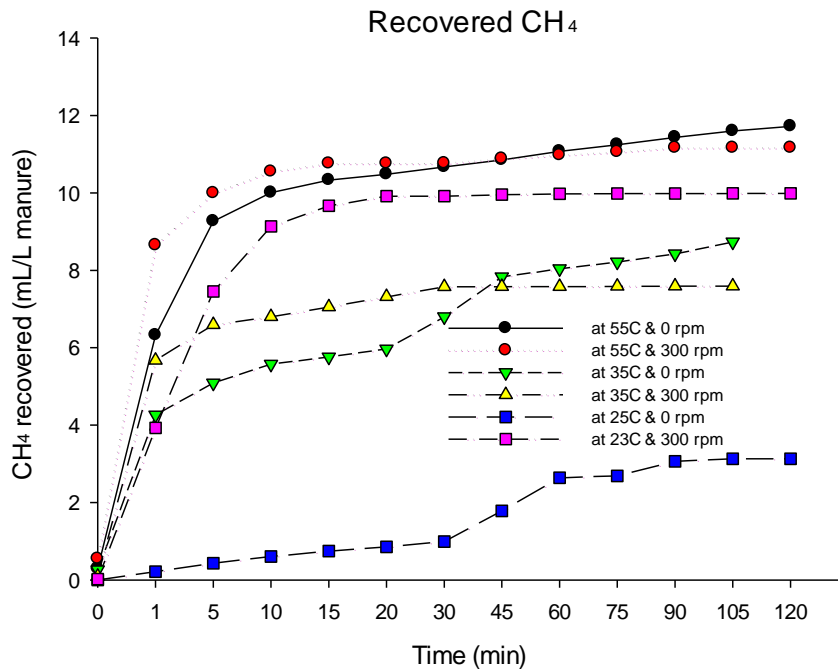


Figure 8.8: D-CH₄ recovery at various temperatures and mixing speeds

The results in Figure 8.8 indicated that higher temperatures significantly improved D-CH₄ recovery due to a lower solubility of gases. However, one must note that high temperatures will lead to higher costs for engineering applications. Mixing of the CSTR also had a positive effect on the removal of D-CH₄. The combination of high temperature (55°C) and high mixing speed (300 rpm) allowed for the greatest overall removal of D-CH₄, but it is worth mentioning that a significant amount of D-CH₄ was also removed at the lower temperature (25°C) and a high mixing speed (300 rpm), which could help with the economics of this recovery process.

Figure 8.9 illustrates the concentrations of D-CO₂ and D-H₂S during the same experiments. Although the solubilities of the two gases may be different, the removal of both these acidic gases should occur once the temperature is increased in the effluent. In addition, agitating the effluent should also increase the release of these dissolved gases. As can be seen Figure 8.9, left, the recovery of D-CO₂ was mainly a function of temperature, with the highest removals occurring at a temperature of 55°C. Interestingly, mixing did little to affect the removal of D-CO₂ since at both a low mixing speed (0 rpm) and high mixing speed (300 rpm) the removals were similar (at a temperature of 55°C). When considering the removal of D-H₂S, the recovery system had little to no effect. There was higher removal at a temperature of 35°C with no mixing but recovery is still extremely low when compared with the removal of D-CH₄ and D-CO₂. This could be due to the fact that the production of H₂S during the anaerobic process is much lower than CH₄ and CO₂,

resulting in a lower D-H₂S. It could also be a reflection of the fact that H₂S has a higher solubility than CO₂ and is less likely to be released from the liquid phase.

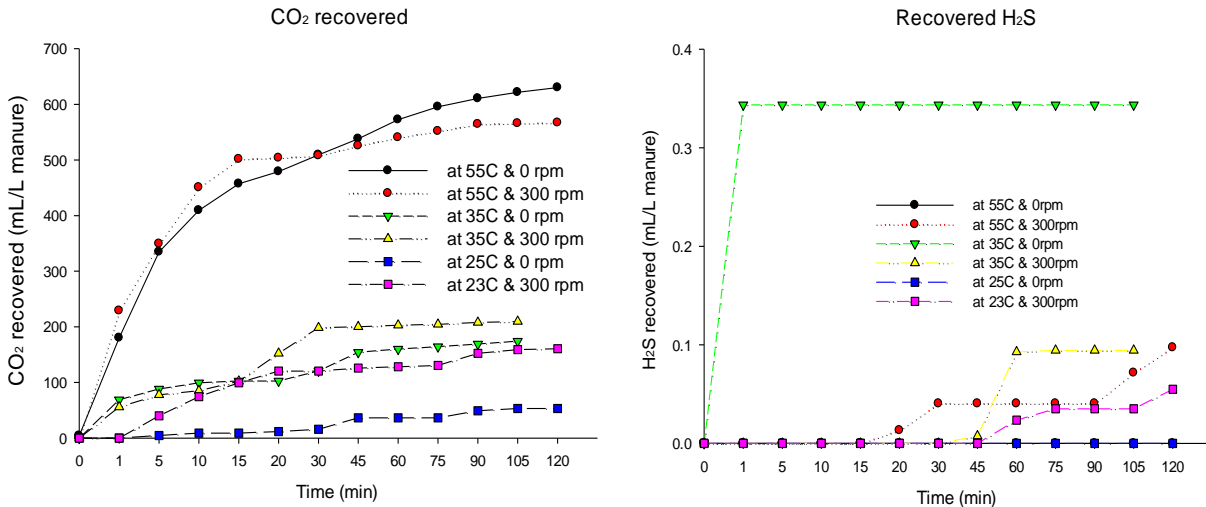


Figure 8.9: Recovery of D-CO₂ (*left*) and D-H₂S (*right*) at various temperatures and mixing speeds

The pH was monitored throughout the experiment to determine how the pH of the effluent was affected by the release of the dissolved gases. As the amount of D-CO₂ in the effluent decreased, the pH of the effluent increased because CO₂ is slightly acidic (Figure 8.10). As can be seen in the figure, the pH of a 1 L of AD effluent solution can be increased from 7.8 to around 9.2 after 2 hrs of degassing via a vacuum pump. This could be helpful for downstream processing such as ammonia stripping (Zhao et al., 2012). In traditional ammonia stripping of digested effluent, increasing pH of the effluent is achieved by adding alkali (Lei et al., 2007), which often hurts the economics of the process. This process could raise the pH to the appropriate levels for downstream ammonia stripping with a lower cost.

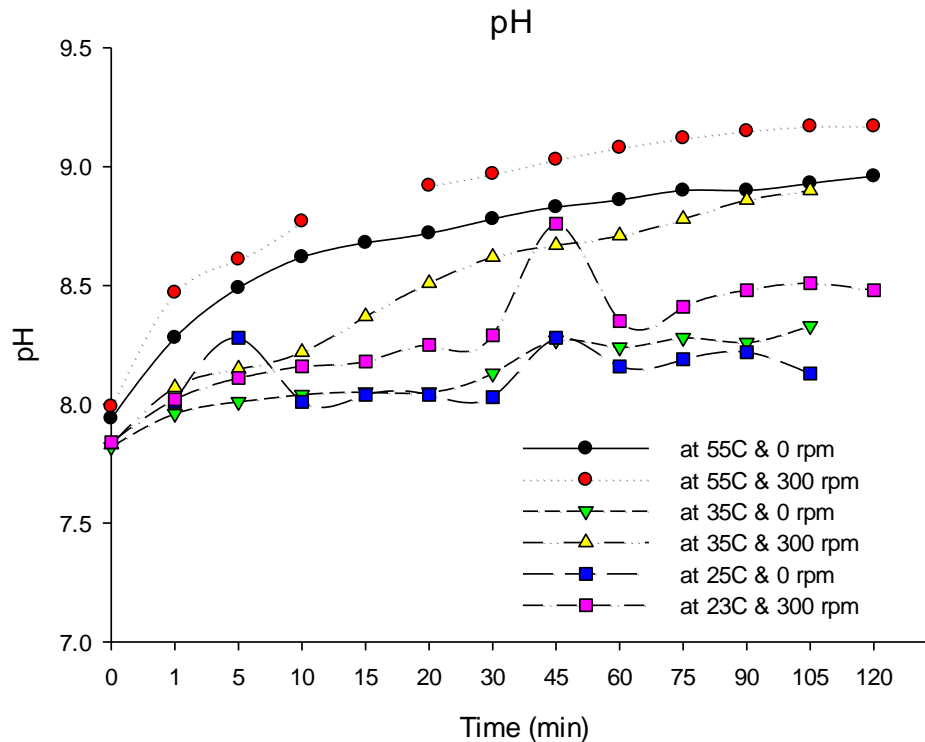


Figure 8.10: pH of the effluent at various temperatures and mixing speeds

8.3.4 Conclusions

The results of this experiment indicated a strong relationship between temperature and the recovery of dissolved gases (primarily CH_4 and CO_2 , but also to a lesser extent H_2S), that can be attributed to the solubility of the gases. As the temperature increased, the concentrations of recoverable D- CH_4 and D- CO_2 increased significantly. Figure 8.8 indicates clearly that the effects of temperature dominate those from mixing, though a future statistical analysis should be performed to gain a better understanding of the influences of these two operational parameters. Our results also showed that removing D- CO_2 and D- H_2S from the effluent raised the pH, which is beneficial for subsequent ammonia stripping if NR is incorporated. In the case of combined AD-NR, degassing the effluent via a vacuum could provide another way to increase the pH of the effluent, lessening the requirements for alkali inputs to remove ammonia from the effluent.

An economic analysis would be beneficial to compare the energy costs required to run this degassing system with the cost of alkali input for traditional ammonia stripping techniques. Such analysis should also seek to determine whether the quantity of CH_4 that was recovered offsets the additional costs for the energy consumption and heat required for the system to work optimally.

References

- Abatzoglou, N., Boivin, S. 2009. A review of biogas purification processes. *Biofuels, Bioproducts and Biorefining*, **3**(1), 42-71.
- Abbasi, T., Tauseef, S.M., Abbasi, S.A. 2012. Anaerobic digestion for global warming control and energy generation—An overview. *Renewable and Sustainable Energy Reviews*, **16**(5), 3228-3242.
- Agler, M.T., Wrenn, B.A., Zinder, S.H., Angenent, L.T. 2011. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends in Biotechnology*, **29**(2), 70-78.
- Agrawal, L.K., Harada, H., Okui, H. 1997. Treatment of dilute wastewater in a UASB reactor at a moderate temperature: Performance aspects. *Journal of Fermentation and Bioengineering*, **83**(2), 179-184.
- AgStar. 2010a. Anaerobic digestion capital costs for dairy farms, EPA. Mankato.
- AgStar. 2010b. Market opportunities for biogas recovery systems-a guide to identifying candidates for on-farm and centralized systems. Washington DC.
- Ageyi, N., Strydom, C., Potgieter, J. 2000. An investigation of phosphate ion adsorption from aqueous solution by fly ash and slag. *Cement and Concrete Research*, **30**(5), 823-826.
- Ageyi, N.M., Strydom, C., Potgieter, J. 2002. The removal of phosphate ions from aqueous solution by fly ash, slag, ordinary Portland cement and related blends. *Cement and Concrete Research*, **32**(12), 1889-1897.
- Aillery, M., Gollehon, N., Johansson, R., Kaplan, J., Key, N., Ribaud, M. 2005. *Managing manure to improve air and water quality*. US Department of Agriculture, Economic Research Service.
- Alva, A., Paramasivam, S., Fares, A., Delgado, J., Mattos Jr, D., Sajwan, K. 2006. Nitrogen and irrigation management practices to improve nitrogen uptake efficiency and minimize leaching losses. *Journal of Crop Improvement*, **15**(2), 369-420.
- Amani, T., Nosrati, M., Sreekrishnan, T.R. 2010. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects - a review. *Environmental Reviews*, **18**, 255-278.
- Archibeque, S.L., Miller, D.N., Freetly, H.C., Berry, E.D., Ferrell, C.L. 2007. The influence of oscillating dietary protein concentrations on finishing cattle. I. Feedlot performance and odorous compound production. *Journal of Animal Science*, **85**(6), 1487-95.
- ASAE. 2005. Manure production and characteristics. American Society of Agricultural Engineers. Michigan, USA.
- Bayer, E.A., Lamed, R., Himmel, M.E. 2007. The potential of cellulases and cellosomes for cellulosic waste management. *Current Opinion in Biotechnology*, **18**(3), 237-45.
- Bektas, N., Akbulut, H., Inan, H., Dimoglo, A. 2004. Removal of phosphate from aqueous solutions by electro-coagulation. *Journal of Hazardous Materials*, **106**(2-3), 101-105.
- Bendall, E., Aiken, R.C., Mandas, F. 1983. Selective absorption of H₂S from larger quantities of CO₂ by absorption and reaction in fine sprays. *AIChE Journal*, **29**(1), 66-72.

- Beszedes, S., Kertesz, S., Laszlo, Z., Szabo, G., Hodur, C. 2009. Biogas production of ozone and/or microwave-pretreated canned maize production sludge. *Ozone Science and Engineering*, **31**(3), 257-261.
- Bishop, C.P., Shumway, C.R. 2009. The economics of dairy anaerobic digestion with coproduct marketing. *Applied Economic Perspectives and Policy*, **31**(3), 394-410.
- Biswas, R., Ahring, B.K., Uellendahl, H. 2012. Improving biogas yields using an innovative concept for conversion of the fiber fraction of manure. *Water Science & Technology*.
- Braun, R., Weiland, P., Wellinger, A. 2010. Biogas from Energy Crop Digestion.
- Bush, R.A., Hall, K.M. 2006. Process for the production of ethanol from algae. in: *Google patents*.
- Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, **8**(3), 559-568.
- CAST. 2002. Animal diet modification to decrease the potential for nitrogen and phosphorus pollution. *Council for Agricultural Science and Technology*(21).
- Chandra, R., Takeuchi, H., Hasegawa, T. 2012. Hydrothermal pretreatment of rice straw biomass: A potential and promising method for enhanced methane production. *APEN Applied Energy*, **94**, 129-140.
- Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*, **25**(3), 294-306.
- Chynoweth, D.P., Turick, C.E., Owens, J.M., Jerger, D.E., Peck, M.W. 1993. Biochemical methane potential of biomass and waste feedstocks. *Biomass and Bioenergy*, **5**(1), 95-111.
- Coppedge, B., Coppedge, G., Evans, D., Jensen, J., Kanoa, E., Scanlan, K., Scanlan, B., Weisberg, P., Frear, C. 2012. Renewable natural gas and nutrient recovery feasibility for DeRuyter Dairy: an anaerobic digester case study for alternative offtake markets and remediation of nutrient loading concerns within the region. Washington State Department of Commerce. Olympia, WA.
- Davis, J.G., Dernoeden, P.H. 2002. Dollar spot severity, tissue nitrogen, and soil microbial activity in bentgrass as influenced by nitrogen source. *Crop Science*, **42**(2), 480-488.
- Davis, R., Aden, A., Pienkos, P.T. 2011. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy*, **88**(10), 3524-3531.
- de Hullu, J., Massen, J.I.W., van Meel, P.A., Shazad, S., Vaessen, J.M.P. 2008. Comparing different biogas upgrading techniques. Eindhoven University of Technology.
- DeBruyn, J., House, H., Rodenburg, J. 2006. Ontario large herd operators european anaerobic digestion tour report. LHO. Ontario.
- Deckwer, W.-D., Field, R.W. 1992. *Bubble column reactors*. Wiley, Chichester; New York.
- Delrue, F., Setier, P.A., Sahut, C., Cournac, L., Roubaud, A., Peltier, G., Froment, A.K. 2012. An economic, sustainability, and energetic model of biodiesel production from microalgae. *Bioresource Technology*, **111**, 191-200.
- Dobermann, A. 2007. Nutrient use efficiency—measurement and management. *Fertilizer Best Management Practices*, 1.
- Eghball, B., Wienhold, B.J., Gilley, J.E., Eigenberg, R.A. 2002. Mineralization of manure nutrients. *Journal of Soil and Water Conservation*, **57**(6), 470-473.
- EPA. 2013. Relation between nitrate in water wells and potential sources in the lower Yakima Valley, Washington. EPA. Yakima, WA.
- Erismann, J., Schaap, M. 2004. The need for ammonia abatement with respect to secondary PM reductions in Europe. *Environmental Pollution*, **129**(1), 159-163.

- Ferguson, R.B., Schepers, J.S., Hergert, G.W., Lohry, R.D. 1991. Corn uptake and soil accumulation of nitrogen: management and hybrid effects. *Soil Science Society of America Journal*, **55**(3), 875-880.
- Fey, A., Conrad, R. 2000. Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Applied and Environmental Microbiology*, **66**(11), 4790-7.
- Fine, P., Hadas, E. 2012. Options to reduce greenhouse gas emissions during wastewater treatment for agricultural use. *Science of The Total Environment*, **416**(0), 289-299.
- Frear, C. 2009. Anaerobic digestion strategies for dairy manures. in: *Biological Systems Engineering*, Vol. Doctor of Philosophy Washington State University. Pullman, pp. 143.
- Frear, C., Chen, S., Wang, Z.W., Li, C. 2011a. Biogas potential and microbial population distributions in flushed dairy manure and implications on anaerobic digestion technology. *Journal of Chemical Technology and Biotechnology*, **86**(1), 145-152.
- Frear, C., Ewing, T., Chen, S., Liao, W. 2011b. Evaluation of co-Digestion at a commercial dairy anaerobic digester. *Clean Soil Air Water Clean - Soil, Air, Water*, **39**(7), 697-704.
- Frear, C., Zhao, B., Fu, G., Richardson, M., Chen, S., Fuchs, M. 2005. Biomass Inventory and Bioenergy Assessment Washington State University. Olympia.
- Fu, Z., Holtzapple, M.T. 2010a. Anaerobic mixed-culture fermentation of aqueous ammonia-treated sugarcane bagasse in consolidated bioprocessing. *Biotechnology Bioengineering*, 216-227.
- Fu, Z., Holtzapple, M.T. 2010b. Consolidated bioprocessing of sugarcane bagasse and chicken manure to ammonium carboxylates by a mixed culture of marine microorganisms. *Bioresource Technology*, **101**(8), 2825-2836.
- Fu, Z., Holtzapple, M.T. 2011. Anaerobic thermophilic fermentation for carboxylic acid production from in-storage air-lime-treated sugarcane bagasse. *Applied Microbiology and Biotechnology*, 1669-1679.
- Galinato, S.P., C.E. Kruger, Frear, C.S. 2013. Economic feasibility of post-digester nutrient recovery using struvite crystallization and WSU AIRTRAP approach.
- Gao, A.H. 2012. Pretreatment of wheat straw with ozone and soaking aqueous ammonia. in: *Biological Systems Engineering*, Vol. M.S. , Washington State University. Pullman.
- Gao, A.H., Bule, M.V., Laskar, D.D., Chen, S. 2012. Structural and thermal characterization of wheat straw pretreated with aqueous ammonia soaking. *Journal of Agricultural and Food Chemistry*, **60**(35), 8632-8639.
- García-Cubero, M.A., González-Benito, G., Indacochea, I., Coca, M., Bolado, S. 2009. Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rye straw. *Bioresource Technology*, **100**(4), 1608-13.
- Garner, F.H., Long, R., Pennell, A. 1958. The selective absorption of hydrogen sulphide in carbonate solutions. *Journal of Applied Chemistry*, **8**(5), 325-336.
- Ghirardi, M. 2000. Microalgae: a green source of renewable H₂. *Trends in Biotechnology*, **18**(12), 506-511.
- Guan, T.Y., Holley, R.A. 2003. Pathogen survival in swine manure environments and transmission of human enteric illness—a review. *Journal of Environmental Quality*, **32**(2), 383-392.
- Hartley, K., Lant, P. 2006. Eliminating non-renewable CO₂ emissions from sewage treatment: An anaerobic migrating bed reactor pilot plant study. *Biotechnology and Bioengineering*, **95**(3), 384-398.

- Hashimoto, A.G. 1989. Effect of inoculum/substrate ratio on methane yield and production rate from straw. *Biological Wastes*, **28**(4), 247-255.
- Hatamoto, M., Miyauchi, T., Kindaichi, T., Ozaki, N., Ohashi, A. 2011. Dissolved methane oxidation and competition for oxygen in down-flow hanging sponge reactor for post-treatment of anaerobic wastewater treatment. *Bioresource Technology*, **102**(22), 10299-10304.
- Hatamoto, M., Yamamoto, H., Kindaichi, T., Ozaki, N., Ohashi, A. 2010. Biological oxidation of dissolved methane in effluents from anaerobic reactors using a down-flow hanging sponge reactor. *Water Research*, **44**(5), 1409-1418.
- He, Y., Pang, Y., Li, X., Liu, Y., Li, R., Zheng, M. 2009. Investigation on the changes of main compositions and extractives of rice straw pretreated with sodium hydroxide for biogas production. *Energy & Fuels*, **23**(4), 2220-2224.
- Heathwaite, L., Sharpley, A., Gburek, W. 2000. A conceptual approach for integrating phosphorus and nitrogen management at watershed scales. *Journal of Environmental Quality*, **29**(1), 158-166.
- Henry, W.R., Seagraves, J.A. 1960. Economic aspects of broiler production density. *Journal of Farm Economics*, **42**(1), 1-17.
- Hess, M., Sczyrba, A., Egan, R., Kim, T.W., Chokhawala, H., Schroth, G., Luo, S., Clark, D.S., Chen, F., Zhang, T. 2011. Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. *Science*, **331**(6016), 463.
- Hill, J., Nelson, E., Tilman, D., Polasky, S., Tiffany, D. 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proceedings of the National Academy of Sciences of the United States of America*, **103**(30), 11206-10.
- Himmel, M.E., Ding, S.Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D., National Renewable Energy Laboratory, G.C.O. 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science*, **315**(9 February 2007), 804-807.
- Holtzapple, M.T., Davison, R.R., Ross, M.K., Aldrett-Lee, S., Nagwani, M., Lee, C.M., Lee, C., Adelson, S., Kaar, W., Gaskin, D. 1999. Biomass conversion to mixed alcohol fuels using the MixAlco process. *Applied Biochemistry and Biotechnology*, **79**(1), 609-631.
- Holtzapple, M.T., Granda, C.B. 2009. Carboxylate platform: the MixAlco process part 1: comparison of three biomass conversion platforms. *Applied Biochemical Biotechnology*, **156**(1-3), 95-106.
- Hudson, N., Duperouzel, D., Melvin, S. 2006. Assessment of permeable covers for odour reduction in piggery effluent ponds. 1. Laboratory-scale trials. *Bioresource Technology*, **97**(16), 2002-2014.
- ICUSD. 2011. *2011 U.S. Dairy Sustainability Report*. Innovation Center for U.S. Dairy.
- Jeppesen, E., Søndergaard, M., Søndergaard, M., Christoffersen, K. 1998. *The structuring role of submerged macrophytes in lakes*. Springer.
- Jones, C.S., Mayfield, S.P. 2012. Algae biofuels: versatility for the future of bioenergy. *Current Opinion in Biotechnology*, **23**(3), 346-51.
- Jungersen, G., Ahring, B.K. 1994. Anaerobic digestion of liquified cow manure pretreated by catalytic liquefaction. *Water Science & Technology*, **30**(12).
- Kantarci, N., Borak, F., Ulgen, K.O. 2005. Bubble column reactors. *Process Biochemistry*, **40**(7), 2263-2283.

- Kanwar, S., Guleri, R. 1994. Performance evaluation of a family-size, rubber-balloon biogas plant under hilly conditions. *Bioresource Technology*, **50**(2), 119-121.
- Kapdi, S.S., Vijay, V.K., Rajesh, S.K., Prasad, R. 2005. Biogas scrubbing, compression and storage: perspective and prospectus in Indian context. *Renewable Energy*, **30**(8), 1195-1202.
- Kashyap, D.R., Dadhich, K.S., Sharma, S.K. 2003. Biomethanation under psychrophilic conditions: a review. *Bioresource Technology*, **87**(2), 147-53.
- Keating, C., Cysneiros, D., Mahony, T., O'Flaherty, V. 2013. The hydrolysis and biogas production of complex cellulosic substrates using three anaerobic biomass sources. *Water Science and Technology*, **67**(2), 293-8.
- Kiely, G. 1997. *Environmental Engineering*. McGrawHill, London.
- Kim, T.H., Gupta, R., Lee, Y.Y. 2009. Pretreatment of biomass by aqueous ammonia for bioethanol production in: *Biofuels: Methods in Molecular Biology*, Vol. 581, pp. 79-91.
- Kim, T.H., Lee, Y.Y. 2006. Fractionation of corn stover by hot-water and aqueous ammonia treatment. *Bioresource Technology*, **97**(2), 224-232.
- Kruger, C., G. Yorgey, S. Chen, H. Collins, C. Feise, C. Frear, D. Granatstein, S. Higgins, D. Huggins, C. MacConnell, K. Painter, Stöckle., C. 2010. Climate friendly farming: improving the carbon footprint of agriculture in the Pacific Northwest. Washington State University.
- Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial & Engineering Chemistry Research*, **48**(8), 3713-3729.
- Lehmann, J. 2007. Bio-energy in the black. *Frontiers in Ecology and the Environment*, **5**(7), 381-387.
- Lehmann, J., Gaunt, J., Rondon, M. 2006. Bio-char sequestration in terrestrial ecosystems—a review. *Mitigation and Adaptation Strategies for Global Change*, **11**(2), 395-419.
- Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D. 2011. Biochar effects on soil biota—a review. *Soil Biology and Biochemistry*, **43**(9), 1812-1836.
- Lei, X., Sugiura, N., Feng, C., Maekawa, T. 2007. Pretreatment of anaerobic digestion effluent with ammonia stripping and biogas purification. *Journal of Hazardous Materials*, **145**(3), 391-397.
- Leschine, S.B. 1995. Cellulose degradation in anaerobic environments. *Annual Review of Microbiology*, **49**, 399-426.
- Lettinga, G., van Velsen, A.F.M., Hobma, S.W., de Zeeuw, W., Klapwijk, A. 1980. Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, **22**(4), 699-734.
- Liao, W., Liu, Y., Liu, C., Wen, Z., Chen, S. 2006. Acid hydrolysis of fibers from dairy manure. *BITE Bioresource Technology*, **97**(14), 1687-1695.
- Liao, W., Liu, Y., Wen, Z., Frear, C., Chen, S. 2008. Kinetic modeling of enzymatic hydrolysis of cellulose in differently pretreated fibers from dairy manure. *Biotechnology and Bioengineering*, **101**(3), 441-451.
- Liebrand, C., Ling, K. 2009. Cooperative approaches for implementation of dairy manure digesters. USDA, Rural Development. Washington, D.C., <http://hdl.handle.net/10113/35107>.

- Liew, L.N. 2011. Solid-state anaerobic digestion of lignocellulosic biomass for biogas production, Vol. Master of Science Ohio State University.
- Lu, S., Bai, S., Zhu, L., Shan, H. 2009. Removal mechanism of phosphate from aqueous solution by fly ash. *Journal of Hazardous Materials*, **161**(1), 95-101.
- Ma, J., Yu, L., Frear, C., Zhao, Q., Li, X., Chen, S. 2013. Kinetics of psychrophilic anaerobic sequencing batch reactor treating flushed dairy manure. *Bioresource Technology*, **131**(0), 6-12.
- Mac Donald, J., Ribaud, M., Livingston, M., Beckman, J., Huang, W. 2009. Manure use for fertilizer and for energy. *Report to Congress.[online].[Dostęp 24.03. 2011 r.]. Dostępny w Internecie: www.ers.usda.gov/publications/AP/AP037fm.pdf.*
- Martin, J., Roos, K. 2007. Comparison of the performance of a conventional and a modified plug-flow digester for scraped dairy manure. *International Symposium on Air Quality and Waste Management for Agriculture. CD-Rom Proceedings of the 16-19 September 2007 Conference (Broomfield, Colorado), ASABE Publication.*
- Massé, D.I., Droste, R.L. 2000. Comprehensive model of anaerobic digestion of swine manure slurry in a sequencing batch reactor. *Water Research*, **34**(12), 3087-3106.
- Mata, T.M., Martins, A.A., Caetano, N.S. 2010. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, **14**(1), 217-232.
- McCubbin, D.R., Apelberg, B.J., Roe, S., Divita, F. 2002. Livestock ammonia management and particulate-related health benefits. *Environment Science & Technology*, **36**(6), 1141-1146.
- Mehta, A. 2002. The economics and feasibility of electricity generation using manure digesters on small and mid-size dairy farms. University of Wisconsin
- Metje, M., Frenzel, P. 2007. Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. *Environmental Microbiology*, **9**(4), 954-64.
- Mitchell, R., Babcock, R., Hirsch, H., McKee, L. 2005. Water quality: abbotsford-sumas final report. Western Washington University.
- Murphy, J.D., McKeogh, E., Kiely, G. 2004. Technical/economic/environmental analysis of biogas utilisation. *Applied Energy*, **77**(4), 407-427.
- Nallathambi Gunaseelan, V. 1997. Anaerobic digestion of biomass for methane production: A review. *Biomass and Bioenergy*, **13**(1-2), 83-114.
- Namasivayam, C., Sangeetha, D. 2004. Equilibrium and kinetic studies of adsorption of phosphate onto ZnCl₂ activated coir pith carbon. *Journal of Colloid and Interface Science*, **280**(2), 359-365.
- Nedwell, D. 1999. Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. *FEMS Microbiology Ecology*, **30**(2), 101-111.
- Nozhevnikova, A.N., Simankova, M.V., Parshina, S.N., Kotsyurbenko, O.R. 2001. Temperature characteristics of methanogenic archaea and acetogenic bacteria isolated from cold environments. *Water Science and Technology*, **44**(8), 41-8.
- NRC. 1993. *Managing wastewater in coastal urban areas*. The National Academies Press.
- Oguz, E. 2005. Sorption of phosphate from solid/liquid interface by fly ash. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **262**(1), 113-117.
- Owen, W.F., Stuckey, D.C., Healy Jr, J.B., Young, L.Y., McCarty, P.L. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research*, **13**(6), 485-492.

- Park, S., Li, Y. 2012. Evaluation of methane production and macronutrient degradation in the anaerobic co-digestion of algae biomass residue and lipid waste. *BITE Bioresource Technology*, **111**, 42-48.
- Parkin, G., Owen, W. 1986. Fundamentals of anaerobic digestion of wastewater sludges. *Journal of Environmental Engineering*, **112**(5), 867-920.
- Patel, V., Desai, M., Madamwar, D. 1993. Thermochemical pretreatment of water hyacinth for improved biomethanation. *Applied Biochemistry and Biotechnology*, **42**(1), 67-74.
- Power, S., Barker, C., Allchin, E., Ashmore, M., Bell, J. 2001. Habitat management: a tool to modify ecosystem impacts of nitrogen deposition? *The Scientific World Journal*, **1**, 714-721.
- Rajeshwari, K.V., Balakrishnan, M., Kansal, A., Lata, K., Kishore, V.V.N. 2000. State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. *Renewable and Sustainable Energy Reviews*, **4**(2), 135-156.
- Ribaud, M., Kaplan, J., Christensen, L.A., Gollehon, N., Johansson, R., Breneman, V., Aillery, M., Agapoff, J., Peters, M. 2003. Manure management for water quality costs to animal feeding operations of applying manure nutrients to land. USDA-ERS
- Rughoonundun, H., Granda, C., Mohee, R., Holtzaple, M.T. 2010. Effect of thermochemical pretreatment on sewage sludge and its impact on carboxylic acids production. *Waste Management*, **30**(8-9), 1614-21.
- Safley, L.M., Westerman, P.W. 1990. Psychrophilic anaerobic digestion of animal manure: Proposed design methodology. *Biological Wastes*, **34**(2), 133-148.
- Samson, R., Leduyt, A. 1982. Biogas production from anaerobic digestion of *Spirulina maxima* algal biomass. *Biotechnology Bioengineering*, **24**(8), 1919-24.
- Samson, R., LeDuyt, A. 1986. Detailed study of anaerobic digestion of *Spirulina maxima* algal biomass. *Biotechnology Bioengineering*, **28**(7), 1014-23.
- Schlegel, A., Dhuyvetter, K., Havlin, J. 1996. Economic and environmental impacts of long-term nitrogen and phosphorus fertilization. *Journal of Production Agriculture*, **9**(1), 114-118.
- Schütz, H., Seiler, W., Conrad, R. 1990. Influence of soil temperature on methane emission from rice paddy fields. *Biogeochemistry*, **11**(2), 77-95.
- Schwarz, W.H. 2001. The cellulosome and cellulose degradation by anaerobic bacteria. *Applied Microbiology and Biotechnology*, **56**(5-6), 5-6.
- Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J., Smith, A.G. 2010. Biodiesel from algae: challenges and prospects. *Current Opinion in Biotechnology*, **21**(3), 277-86.
- Sharpe, R.R., Harper, L.A., Byers, F.M. 2002. Methane emissions from swine lagoons in Southeastern US. *Agriculture, Ecosystems & Environment*, **90**(1), 17-24.
- Sialve, B., Bernet, N., Bernard, O. 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances*, **27**(4).
- Singh, K.S., Viraraghavan, T. 1998. Start-up and operation of UASB reactors at 20°C for municipal wastewater treatment. *Journal of Fermentation and Bioengineering*, **85**(6), 609-614.
- Souza, C.L., Chernicharo, C.A., Aquino, S.F. 2011. Quantification of dissolved methane in UASB reactors treating domestic wastewater under different operating conditions. *Water Science and Technology*, **64**(11), 2259-2264.

- Speece, R.E. 1996. *Anaerobic biotechnology for industrial wastewaters*. Archae Press, Nashville, Tenn.
- Srinivasan, V., Aiken, R.C. 1988. Selective absorption of H₂S from CO₂ -- factors controlling selectivity toward H₂S. *Fuel Processing Technology*, **19**(2), 141-152.
- Sutton, M., Flower, D., Moncriefe, J. 1993. The exchange of atmospheric ammonia with vegetated surfaces. I: Unfertilized vegetation. *Quarterly Journal of the Royal Meteorological Society*, **119**(513), 1023-1045.
- Uellendahl, H., Ahring, B.K. 2010. Anaerobic digestion as final step of a cellulosic ethanol biorefinery: Biogas production from fermentation effluent in a UASB reactor-pilot-scale results. *Biotechnology and Bioengineering*, **107**(1), 59-64.
- Ullman, J.L., Mukhtar, S. 2007. Impact of dairy housing practices on lagoon effluent characteristics: Implications for nitrogen dynamics and salt accumulation. *Bioresource Technology*, **98**(4), 745-752.
- US-EPA. 2004. National emission inventory - ammonia emissions from animal husbandry. U.S. Environmental Protection Agency, Washington, DC.
- US-EPA. 2012. *Relation between nitrate in water wells and potential sources in the Lower Yakima Valley, Washington*. U.S. EPA Region 10.
- USDA-ERS. 2011. Nitrogen in Agricultural Systems: Implications for Conservation Policy.
- VanderZaag, A.C., Wagner-Riddle, C., Park, K.H., Gordon, R.J. 2011. Methane emissions from stored liquid dairy manure in a cold climate. *Animal Feed Science and Technology*, **166-167**(0), 581-589.
- Vanotti, M.B., Bundy, L.G. 1994. Corn nitrogen recommendations based on yield response data. *Journal of Production Agriculture*, **7**(2), 249-256.
- Viers, J., Liptzin, D., Rosenstock, T., Jensen, V., Hollander, A., McNally, A., King, A., Kourakos, G., Lopez, E., Mora, N.D.L., FryjoffHung, A., Dzurella, K., Canada, H., Laybourne, S., McKenney, C., Darby, J., Quinn, J., Harter, T. 2012. Technical report 2: nitrogen sources and loading to groundwater UC Davis. Davis.
- Wallin, M., Olausson, S. 1993. Simultaneous absorption of H₂S and CO₂ into a solution of sodium carbonate. *Chemical Engineering Communications*, **123**(1), 43 - 59.
- Wang, Q.H., Kuninobu, M., Kakimoto, K., I-Ogawa, H., Kato, Y. 1999. Upgrading of anaerobic digestion of waste activated sludge by ultrasonic pretreatment. *Bioresource Technology*, **68**(3), 309-313.
- Wang, Z.-W., Ma, J., Chen, S. 2011. Bipolar effects of settling time on active biomass retention in anaerobic sequencing batch reactors digesting flushed dairy manure. *Bioresource Technology*, **102**(2), 697-702.
- Ward, M., deKok, T., Levallois, P., Brender, J., Gulis, G., Nolan, B., VanDerslice, J. 2005a. Workgroup report: drinking-water nitrate and health—recent findings and research needs. *Environment Health Perspective*, **11**(113).
- Ward, M.L., Bitton, G., Townsend, T. 2005b. Heavy metal binding capacity (HMBC) of municipal solid waste landfill leachates. *Chemosphere*, **60**(2), 206-215.
- Weiland, P. 2006. Biomass digestion in agriculture: a successful pathway for the energy production and waste treatment in germany. *Engineering in Life Sciences*, **6**(3), 302-309.
- Weimer, P.J., Russell, J.B., Muck, R.E. 2009. Lessons from the cow: What the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass. *Bioresource Technology*, **100**(21), 5323-5331.

- Wilkie, A.C., Castro, H.F., Cubinski, K.R., Owens, J.M., Yan, S.C. 2004. Fixed-film anaerobic digestion of flushed dairy manure after primary treatment: wastewater production and characterisation. *Biosystems Engineering*, **89**(4), 457-471.
- WSDA. 2006. Small-scale dairies: barriers to licensing. WSDA. Olympia, WA. http://www.sos.wa.gov/library/docs/agr/2006WSDASmDairyLegReportFINAL12-06_2007_001301.pdf.
- WSDA. 2011. Washington dairies and digesters. Olympia, WA.
- Xue, Y., Hou, H., Zhu, S. 2009. Characteristics and mechanisms of phosphate adsorption onto basic oxygen furnace slag. *Journal of Hazardous Materials*, **162**(2), 973-980.
- Yen, H., Brune, D. 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource Technology*, **98**(1), 130-134.
- Yih, S.-M., Sun, C.-C. 1987. Simultaneous absorption of hydrogen sulfide and carbon dioxide into di-isopropanolamine solution. *The Canadian Journal of Chemical Engineering*, **65**(4), 581-585.
- Yue, Z., Teater, C., MacLellan, J., Liu, Y., Liao, W. 2011. Development of a new bioethanol feedstock - Anaerobically digested fiber from confined dairy operations using different digestion configurations. *JBB Biomass and Bioenergy*, **35**(5), 1946-1953.
- Zamalloa, C., De Vrieze, J., Boon, N., Verstraete, W. 2011. Anaerobic digestibility of marine microalgae *Phaeodactylum tricornutum* in a lab-scale anaerobic membrane bioreactor. *Applied Microbiology Biotechnology*.
- Zhao, Q., Dvorak, S.W., Chen, S., Frear, C., VanLoo, B.J. 2012. Nutrient recovery systems and methods, Vol. US 2012/0118035 A1. USA.